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ABOUT COVER Editorial Board Member of *World Journal of Cardiology*, Abdallah Al-Mohammad, MD, MRCP, Doctor, Cardiology Department, Northern General Hospital, Sheffield S5 7AU, United Kingdom

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Etiology of bicuspid aortic valve disease: Focus on hemodynamics

Samantha K Atkins, Philippe Sucosky

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Abstract

The bicuspid aortic valve (BAV) is the most common form of inheritable cardiac defect. Although this abnormality may still achieve normal valvular function, it is often associated with secondary valvular and aortic complications such as calcific aortic valve disease and aortic dilation. The clinical significance and economic burden of BAV disease justify the need for improved clinical guidelines and more robust therapeutic modalities, which address the root-cause of those pathologies. Unfortunately, the etiology of BAV valvulopathy and aortopathy is still a debated issue. While the BAV anatomy and its secondary complications have been linked historically to a common genetic root, recent advances in medical imaging have demonstrated the existence of altered hemodynamics near BAV leaflets prone to calcification and BAV aortic regions vulnerable to dilation. The abnormal mechanical stresses imposed by the BAV on its leaflets and on the aortic wall could be transduced into cell-mediated processes, leading ultimately to valvular calcification and aortic medial degeneration. Despite increasing evidence for this hemodynamic etiology, the demonstration of the involvement of mechanical

abnormalities in the pathogenesis of BAV disease requires the investigation of causality between the blood flow environment imposed on the leaflets and the aortic wall and the local biology, which has been lacking to date. This editorial discusses the different hypothetical etiologies of BAV disease with a particular focus on the most recent advances in cardiovascular imaging, flow characterization techniques and tissue culture methodologies that have provided new evidence in support of the hemodynamic theory.

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Key words: Aortopathy; Valvulopathy; Hemodynamics; Bicuspid aortic valve; Shear stress

Core tip: The bicuspid aortic valve (BAV) is associated with secondary aortopathy and valvulopathy. However, the root cause of those complications remains controversial. While the genetic etiology has been the most popular historically, advances in cardiovascular imaging, flow characterization and tissue culture methodologies have provided new evidence in support of a hemodynamic origin. The assessment of the respective role of genetic and hemodynamic cues in BAV pathogenesis is critical to the development of improved diagnosis tools and patient-specific modalities. This editorial discusses the different possible etiologies of BAV disease with a particular focus on the most recent evidence for the hemodynamic pathway.

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INTRODUCTION

Despite a limited prevalence of 1%-2% in the general

population^[1-3], the bicuspid aortic valve (BAV) is the most common inheritable valvular defect. As compared to a normal tricuspid aortic valve (TAV), which consists of three leaflets, the BAV forms with only two as a result of cusp fusion during development. The BAV exists in different morphologic phenotypes^[4-6]. The most prevalent type- I morphology features two cusps of unequal size and a fibrous raphe at the location of congenital fusion^[7-9]. While 71% of type- I BAVs result from the fusion between the right- and left-coronary leaflets (LR subtype), 15% feature right- and non-coronary cusp fusion (RN subtype) and 3% present with non- and left-coronary cusp fusion (NL subtype)^[7]. The BAV has emerged as the most common indication for surgical valvular replacement and is often complicated by secondary valvular and aortic wall abnormalities such as calcific aortic valve disease (CAVD) and aortic dilation, respectively^[3,10-17]. The current management of BAV disease presents significant challenges related to the nontrivial early identification of BAV patients, the difficult detection of the onset of aortic and valvular complications and the time-sensitivity of surgical intervention^[18]. The development of improved clinical guidelines and more robust therapeutic modalities addressing the root-cause of BAV disease requires the knowledge of the etiology of those pathologies, which is still under debate. This editorial discusses recent clinical and bioengineering developments in support of the hemodynamic theory of BAV disease, future research needs and their potential impact on clinical management.

BICUSPID AORTIC VALVE DISEASE

CAVD

Formerly considered a passive age-related disease promoted by cardiovascular risk factors and genetic predispositions, CAVD is now recognized as an active disease process involving inflammatory, extracellular matrix remodeling and osteogenic mediators as well as phenotypic changes in the valve interstitial cell population^[19-22]. The later stage is characterized by formation of calcium nodules preferentially on the fibrosa (*i.e.*, leaflet aortic surface)^[23]. The stenosis caused by the stiffening of the leaflet tissue imposes a pressure overload on the left ventricle, which may result in hypertrophy and ultimately lead to heart failure^[24]. While those disease features are common to both TAVs and BAVs, the valve anatomy is a strong predictor of the prevalence and progression of the disease. In the TAV population, CAVD affects about 25% of individuals above 65 years of age and the progression of the disease is relatively slow (20-30 years before severe valvular stenosis can be detected)^[20,25-27]. In contrast, it is estimated that 40%-53% of BAV patients develop some form of CAVD and that it may take as little as 10-12 years for the disease to result in severe valvular stenosis^[16,17].

Aortic dilation

Aortic dilation is another common complication in BAV patients^[10,14,28-31]. This condition, which characterizes the gradual thinning of the aortic wall and the enlargement of the aortic lumen above 4.0 cm in diameter^[32], is a precursor

event to dissection and ultimate rupture. The dilation of the thoracic ascending aorta downstream of a BAV is marked by structural wall abnormalities including aortic medial degradation, smooth muscle cell apoptosis and depletion, elastic fiber degeneration and abnormal extracellular remodeling^[31], which localize primarily to the convexity of the aortic wall^[33,34]. The particular BAV morphotype has also been shown to affect the pattern of dilation. The LR type-I BAV subtype is associated with a larger annulus and sinus than the RN phenotype^[35]. While aortic dilation generally spares TAV patients and only occurs in 12%-22% of those with hypertension^[36,37], it affects between 35%-68% of BAV patients^[11,16,17,31,38-41]. In addition, while TAV ascending aortas typically experience a dilation rate of 0.07-0.2 mm/year, BAV patients experience much more rapid progression of dilation at rates of 0.2-1.9 mm/year^[42,43]. As a result, acute aortic dissection occurs 5 to 10 times more frequently and at an earlier age in BAV patients than in TAV patients^[3,10,44-46].

HYPOTHETICAL ETIOLOGIES AND KNOWLEDGE GAP

While the genetic root of the BAV malformation has been clearly demonstrated, the etiology of BAV disease is still a matter of debate^[47-49]. The most accepted genetic theory hypothesizes that the abnormal valve structure, the vulnerability of BAV leaflets to calcification and the BAV aorta to dilation originate from a common congenital defect. Supporting evidence for this etiology stems from the apparent heritability of the BAV defect^[50,51] and the association between certain gene mutations (*e.g.*, *GATA5*, *NOTCH1*, *ACTA2*), leaflet calcification and aortic dilation^[52-55].

The less popular hemodynamic theory considers the mechanical stresses produced by the abnormal valve anatomy as the driving factor of secondary valvulopathy and aortopathy. Evidence for this mechano-etiology is supported by the apparent correlation between the eccentric BAV orifice jet^[56-59], the presentation of aortic dilation in wall regions subjected to wall shear stress overload^[33,34] and the preferential formation of calcific nodules on the fused BAV leaflet, which experiences a higher degree of wall shear stress abnormality relative to the non-fused leaflet^[3-5,60]. Despite those observations, the validation of the hemodynamic etiology of BAV disease requires demonstration of causality, which to date has been lacking.

EVIDENCE FOR A HEMODYNAMIC PATHWAY

The mechanistic elucidation of the role played by hemodynamics in BAV disease requires the implementation of integrative approaches that not only describe the genetics of valvular morphogenesis and the impact of the BAV anatomy on valvular function but also investigate the adaptive and pathological responses of valvular and aortic cells to the native BAV flow environment. The emergence of state-of-the-art flow measurement and modeling tools combined with advanced tissue conditioning systems have

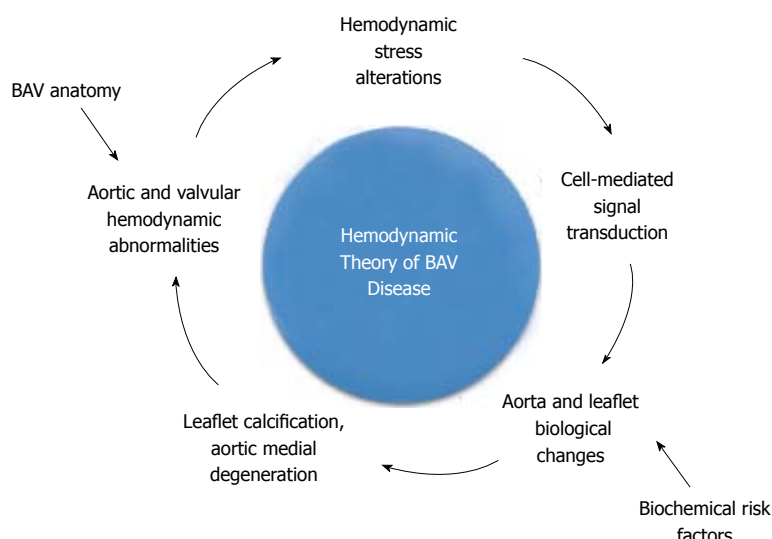


Figure 1 Hemodynamic theory of bicuspid aortic valve disease. The hemodynamic theory of bicuspid aortic valve (BAV) disease can be illustrated as an irreversible feedback loop in which the BAV anatomy would subject the valve leaflets and the ascending aortic wall to local stress overloads. Those stress abnormalities could be sensed by specific endothelial receptors and then transduced into different biological responses that would lead ultimately to the formation of calcific lesions on the leaflets or to the progressive degeneration of the aortic media.

provided a unique opportunity to examine *ex vivo* the role played by BAV hemodynamics, in the absence of underlying genetic defects and concurrent risk factors.

The implementation of advanced clinical and engineering tools toward the quantification of BAV flow has provided new insights into the hemodynamic complexity of this valvular defect and detailed maps of the wall shear stress abnormalities experienced by BAV leaflets and the BAV ascending aorta. *In vitro* particle-image velocimetry measurements in porcine valve models demonstrated the existence of an elliptical valve orifice, an eccentric jet skewed toward the non-coronary leaflet and an intrinsic degree of stenosis in type-I BAVs^[61-63]. Pulsatile fluid-structure interaction simulations in valve and ascending aorta models^[64,65], phase-contrast magnetic resonance imaging^[59,66] and cardiovascular magnetic resonance^[67,68] isolated dramatic differences in the frequency and magnitude of the wall shear stress generated on the leaflets and ascending aorta downstream of a TAV and a type-I BAV. Those modalities identified the fused BAV leaflet and the convex region of the BAV ascending aorta as those experiencing the highest degree of wall shear stress abnormality as compared to their TAV counterparts.

The concurrent development of sophisticated tissue culture systems capable of subjecting native BAV leaflets^[69] or aortas^[70] to their native local hemodynamic stress environment has enabled the rigorous investigation of the isolated effects of BAV flow on valvular calcification and aortic dilation. Those mechanobiological studies demonstrated for the first time: (1) the ability of the wall shear stress overload present on the convexity of LR type-I BAV ascending aortas to promote locally aortic medial degradation *via* matrix metalloproteinase-dependent pathways^[65,71]; and (2) the particular susceptibility of the wall shear stress generated on the fused LR type-I BAV leaflet to trigger early calcification events such as endothelial activation, paracrine signaling, extracellular matrix degradation and bone matrix synthesis^[72,73].

Collectively, those observations support a hemodynamic etiology by which the abnormal mechanical stresses experienced by BAV leaflets and BAV ascending aortas

could trigger molecular pathways leading to the progressive calcification of the leaflets and the weakening of the aortic wall. Specifically, the hemodynamic theory postulates that the abnormal BAV anatomy subjects the valve leaflets and the ascending aortic wall to local stress overloads, which can be sensed by specific receptors in the tissue endothelium and then transduced into different pathological responses that would lead ultimately to the formation of calcific nodules on the leaflets or the progressive degeneration of the aortic media (Figure 1). The amplification of the degree of hemodynamic abnormality caused by the gradual stiffening of the leaflets and dilation of the proximal aorta may result in turn in the amplification of the pathological cascade and the acceleration of the disease process.

POTENTIAL CLINICAL IMPACTS

Whether and how hemodynamic cues contribute to BAV disease are important research questions that need to be addressed thoroughly in order to design improved diagnostic tools and more effective practice guidelines. As recognized by the Heart, Lung and Blood Institute (National Institutes of Health), the current state of the science on BAV disease does not permit to support particular pharmacological targets^[74]. The effectiveness of the pharmacological approach depends on the ability to identify target molecules involved in the early stage of BAV disease before calcification and aortic medial degradation attain a point of no return. Therefore, the elucidation of the role played by hemodynamics in BAV valvulopathy and aortopathy may become instrumental to the future development of targeted cellular therapies as it has the potential to identify candidate mechano-sensitive molecules that may play a significant role in the initiation of BAV disease.

The current practice guidelines for the management of BAV complications^[75] have been developed based on the prevailing theory of their etiology, which, historically, has been the genetic theory^[47,48,53]. The limited understanding of the pathogenesis of BAV calcification and aortic dilation combined with the lack of pharmacological targets has driven the development of aggressive surgical procedures

aimed at recovering valvular function^[76,77] and eliminating the weakened ascending aortic wall^[78,79]. While such strategies may be appropriate to address a congenital disease, it may not be as effective should the formation of valvular calcific lesions or the structural degeneration of the aortic wall be the result of adaptive mechanisms to the abnormal BAV flow. In this context, the involvement of flow-mediated signaling pathways in BAV aortopathy and valvulopathy should not be ignored as they may guide the development of new therapeutic modalities aimed at normalizing BAV flow or inhibiting pharmacologically the valvular and aortic pathological cascades at an early age.

Lastly, current diagnosis techniques for BAV calcification and aortic dilation rely on criteria that can only be assessed at an advanced stage of the disease. The exploration of improved techniques enabling early detection in young patients requires the fundamental knowledge of the disease mechanisms. More importantly, the modeling of those mechanisms in individual patients could provide predictive capabilities that will transform clinical decision-making and personalized care. Therefore, the demonstration of cause-and-effects relationships between BAV hemodynamics, valvular calcification and aortic wall degeneration may lay the foundations for computer-based predictive models of BAV disease by integrating the mathematical formulation of flow-sensitive valvular and vascular biological pathways in patient-specific flow models. Such models will help predict disease onset and progression and guide the choice of the optimal treatment strategy.

FUTURE DIRECTIONS

The novel therapeutic perspectives discussed above suggest several recommendations for future research. The increasing need for understanding the pathogenesis of BAV disease motivates the investigation of potential links between BAV morphotype, genotype and hemodynamics, and the detailed description of the key features that stimulate BAV calcification and aortic dilation.

In addition, new emphasis should be put toward the elucidation of the basic biology of BAV disease from early events to long-term mechanisms. Those efforts would involve the characterization of the signaling pathways of BAV calcification and aortic dilation, as well as the multi-scale description of the synergistic effects between valvular calcification, aortic medial degeneration, micro-scale mechanotransduction and macro-scale hemodynamics.

The current evidence in support of the hemodynamic theory has been provided by short-term *ex vivo* studies and small-scale clinical investigations. The development of improved laboratory methodologies enabling prolonged tissue exposure to BAV hemodynamics while maintaining tissue sterility and integrity may shed some light on how the acute adaptive mechanisms reported thus far evolve in the long-term. Clinical investigations on large BAV patient populations will also permit to determine the validity of the hemodynamic and genetic theories in a more statistically significant way.

CONCLUSION

In summary, evidence for the causative effects of BAV hemodynamics on secondary valvulopathy and aortopathy is emerging. While those complications may still be promoted by some genetic predispositions, it is likely that their pathogenesis is also driven by synergies between the local mechanical stress abnormalities and the local biology of the leaflets and ascending aortic wall. Long-term *ex vivo* studies and large-scale clinical investigations are needed to assess the respective contribution of the genetic and hemodynamic pathways and to determine the full spectrum of mechano-sensitive processes triggered by BAV hemodynamics. The new knowledge gained from those efforts may enable the development of improved diagnosis tools and therapeutic modalities capable of addressing the root cause of BAV disease.

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WJC 6th Anniversary Special Issues (3): Cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy: From genetics to diagnostic and therapeutic challenges

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criteria on the Revised Task Force Criteria. Implantable cardioverter defibrillators (ICDs) are increasingly utilized in patients with ARVC who have survived sudden death (SD) (secondary prevention). However, there are few data available to help identifying ARVC patients in whom the prophylactic implantation of an ICD is truly warranted. Prevention of SD is the primary goal of management. Pharmacologic treatment of arrhythmias, catheter ablation of ventricular tachycardia, and ICD are the mainstay of treatment of ARVC.

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Key words: Arrhythmogenic right ventricular cardiomyopathy; Sudden cardiac death; Risk stratification; Genetic; Implantable cardioverter-defibrillator

Abstract

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetic disease characterized by myocyte loss and fibro-fatty tissue replacement. Diagnosis of ARVC remains a clinical challenge mainly at its early stages and in patients with minimal echocardiographic right ventricular (RV) abnormalities. ARVC shares some common features with other cardiac diseases, such as RV outflow ventricular tachycardia, Brugada syndrome, and myocarditis, due to arrhythmic expressivity and biventricular involvement. The identification of ARVC can be often challenging, because of the heterogeneous clinical presentation, highly variable intra- and inter-family expressivity and incomplete penetrance. This genotype-phenotype "plasticity" is largely unexplained. A familial history of ARVC is present in 30% to 50% of cases, and the disease is considered a genetic cardiomyopathy, usually inherited in an autosomal dominant pattern with variable penetrance and expressivity; in addition, autosomal recessive forms have been reported (Naxos disease and Carvajal syndrome). Diagnosis of ARVC relies on a scoring system, with major or minor

Core tip: This manuscript constitutes an update on arrhythmogenic right ventricular cardiomyopathy (ARVC). Recently, molecular genetic studies have provided significant advances in the understanding the pathogenesis of ARVC. However, criteria on treatment with Implantable cardioverter defibrillators are still lacking. We believe that this topic can provide a useful instrument to physicians and guide them in their clinical practice.

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INTRODUCTION: DEFINITION OF DISEASE, EPIDEMIOLOGY, AND CLINICAL FEATURES

Arrhythmogenic right ventricular cardiomyopathy/dysplasia

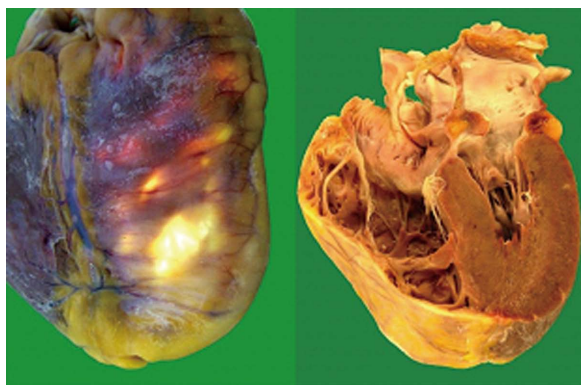


Figure 1 Gross anatomic specimens in a patient affected by arrhythmogenic right ventricular cardiomyopathy who died suddenly. Severe right ventricular enlargement and wall atrophy and fatty replacement are evident.

(ARVC) is an inherited cardiomyopathy (CMP) characterized by fibro-fatty replacement of the right ventricular (RV) myocardium (Figures 1 and 2) that predisposes patients to life-threatening ventricular arrhythmias and slowly progressive ventricular dysfunction^[1-4]. Biventricular and left-dominant forms of the disease are increasingly recognized^[5-7].

The estimated prevalence of ARVC in the general population ranges from 1 in 2000 to 1 in 5000 individuals; men are more frequently affected than women, with an approximate ratio of 3:1^[8]. ARVC is a leading cause of sudden cardiac death (SCD) in young people and in athletes, accounting for up to 10% of deaths from undiagnosed cardiac disease in patients less than 65 years old^[2,9-11]. In particular, in young adults and athletes, ARVC has been reported as the second most frequent cause of SCD^[11]. The disease expression is variable and the penetrance (the proportion of carriers manifesting the disease) appears age-related. According to Dalal *et al*^[12], the median age at onset of the disease is 29 years, whereas it rarely manifests before the age of 12 or after the age of 60 years. The most common presenting symptoms are palpitations and syncope, found in 27% and 26% of patients, respectively. Importantly, life-threatening ventricular arrhythmias and SCD can be the first presentation of the disease^[2,9-11].

ARVC has frequently a progressive course. In the early stage of the disease, structural changes may be absent or subtle and confined to a localized region of the RV. The 3 most common locations of the disease are: the anterior infundibulum, RV apex and subtricuspid infero-basal aspect of the RV, comprising the so-called “triangle of dysplasia”, considered a hallmark of ARVC^[1]. ARVC leads to RV dilatation or aneurysms. With disease progression, further involvement of the RV free wall, and left ventricular (LV) involvement can occur^[13-15].

The natural history of ARVC, in its classic “right dominant” form, has been classified into 4 distinct phases with progressive development of symptoms and structural abnormalities^[3]: (1) concealed phase: a subclinical asymptomatic phase with mild or absence of identifiable structural RV abnormalities. SCD may still occur in this stage of disease^[2,3,10,11]; (2) overt electrical disorder: with palpitations, syncope and typically with

symptomatic ventricular arrhythmias of RV origin usually triggered by effort. Arrhythmias may vary from premature ventricular beats, to non-sustained ventricular tachycardia with left bundle branch block (LBBB) morphology up to ventricular fibrillation leading to cardiac arrest; (3) RV failure: progressive loss of RV myocardium due to fibro-fatty replacement impairs RV function and may result in pump failure; and (4) biventricular failure: an advanced stage with involvement of the interventricular septum and LV causing congestive heart failure (HF). Endocavitary mural thrombosis may occur, especially within RV aneurysm or in the atria if atrial fibrillation is present. The phenotype may eventually resemble an advanced dilated CMP, making the differential diagnosis difficult at this stage.

The identification of ARVC can be often challenging, because of the heterogeneous clinical presentation, highly variable intra- and inter-family expressivity and incomplete penetrance. This genotype-phenotype “plasticity” is largely unexplained. The frequent involvement of the LV^[7,15], sometimes predominant, suggests that ARVC is not a unique entity, but a complex heterogeneous disease with a spectrum of phenotypes and three possible patterns of expression: the *classic* (39% of cases), the *left dominant* (5%) and the *biventricular* (56%) forms^[5]. Consequently, according to some Authors, in this disease it may be more appropriate to use the term of “arrhythmogenic cardiomyopathy” instead of the more “restrictive” ARVC terminology^[6], (see below, section “spectrum of disease”).

ETIOPATHOGENESIS AND GENETICS

A familial history of ARVC is present in 30% to 50% of cases, and the disease is considered a genetic CMP, usually inherited in an autosomal dominant pattern with variable penetrance and expressivity; in addition, autosomal recessive forms have been reported (Naxos disease and Carvajal syndrome)^[16]. Its presumed pathomechanism is presently thought an inherited abnormality of myocytes adhesion caused by defects at the intercellular junctions, at the level of desmosomes, adherens junctions or gap junctions, together comprising the intercalated discs^[17-21]. The role of other non-desmosomal genes is less well established^[18].

The desmosomes have a complex structure that includes several families of adhesion molecules, as the cadherins (desmoglein-DSG and desmocollin-DSC), plakins [desmoplakin-desmoplakin (DSP)], and catenins (plakophilin-PKP, and plakoglobin-JUP). Their main functional role is to link intermediate filaments of the intramyocellular cytoskeleton to the extracellular desmosomal cadherins^[21-23]. Mutations in several genes encoding proteins of the desmosome have been identified in ARVC, the majority of which are located in 5 genes: plakophilin-2 (PKP2), DSP, desmoglein-2 (DSG2), desmocollin-2 (DSC2) and plakoglobin (JUP), the last one causing the autosomal recessive ARVC (Naxos disease)^[16,24].

More uncommonly, ARVC has been related to mutations in other non-desmosomal genes, as transforming growth factor β -3, cardiac ryanodine receptor, trans-membrane protein 43 (TMEM43), tumor protein p63 (TP63), desmin,

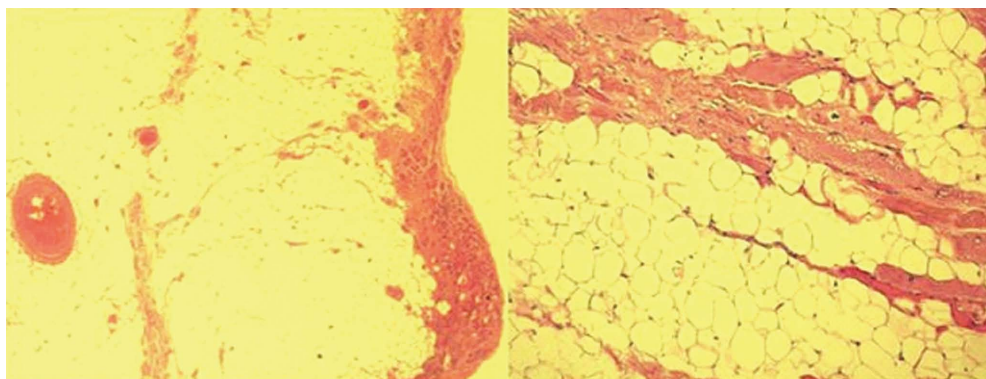


Figure 2 Histologic specimens of a case with arrhythmogenic right ventricular cardiomyopathy that show severe right ventricular fibro-fatty replacement and loss and degeneration of myocytes (Hematoxylin Eosin $\times 2.5, \times 10$).

lamin A/C (*LMNA*), alpha T-catenin (*CTNNA3*) and phospholamban^[17,18]. Thus far, more than 800 genetic variants have been identified in 12 genes, although only around 300 of them have been classified as clearly pathogenic^[17,18]. One review that analyzed pooled data from major ARVC studies noted an overall mutation detection rate of 39.2%^[24]. The most frequently affected gene is *PKP2* with a reported detection rate of 10%-45%^[25,26].

Recently, Taylor *et al*^[27] identified in 7 out of 38 ARVC families an ARVC overlap syndrome due to rare variants in the gene encoding the sarcomeric protein titin (*TTN*), the largest gene in mammals. The phenotype of *TTN* variant carriers was characterized by a frequent history of SCD (5 of 7 families), progressive myocardial dysfunction causing death or heart transplantation (8 of 14 cases), frequent conduction disease (11 of 14), and incomplete penetrance (86%)^[27]. *TTN* filaments bridge the sarcomere along its longitudinal axis, overlapping end-to-end at the Z disc and M band at the amino and carboxyl ends, respectively, thus forming a contiguous filament along the myofibril. Interestingly, recent research showed that *TTN* is involved in cellular mechanics, specifically, in the “spring-like” properties of the sarcomere that underlie passive and restorative forces occurring after sarcomere lengthening or shortening^[28-30]. In this ARVC “overlap” syndrome structural impairment of *TTN* probably leads to proteolysis and apoptosis, which could be hypothesized as a novel mechanism underlying myocardial remodeling and SCD.

SPECTRUM OF DISEASE (CLASSIC ARVC, BIVENTRICULAR, LEFT DOMINANT)

The well known “classic pattern” of ARVC is characterized by an increased RV to LV volume ratio and a more severe involvement of the RV, with LV involvement as a possible late complication of the disease^[1-6]. Clinical hallmarks are negative anterior T waves, and ventricular arrhythmias with LBBB morphology.

Left-dominant arrhythmogenic cardiomyopathy (LDAC) is a novel entity recently described. LDAC is characterized by fibro-adipose replacement, which predominantly involves the LV and often occurs as a circumferential band in the

outer one-third of the myocardium and the right side of the interventricular septum^[7]. This CMP has a predominant (but not necessarily exclusive) LV involvement, characterized by one or more of the following: LV wall motion abnormalities, chamber dilation, systolic impairment, and late gadolinium enhancement (LGE)^[7]. Relevant clinical features of LDAC include ventricular arrhythmias of right bundle branch block (RBBB) morphology, and (infero)-lateral T-wave inversion at electrocardiogram (ECG). According to Sen-Chowdhry *et al*^[6,7], LDAC can be considered one of the three possible patterns of the spectrum of ARVC, together with the “classical” form and the “biventricular” form, in consideration of the histopathologic and genetic similarities. However, as alternative hypothesis, LDAC could be considered as a novel distinct CMP.

Biventricular arrhythmogenic cardiomyopathy

The biventricular subtype of arrhythmogenic CMP is defined by early and parallel involvement of the RV and LV^[6]. While milder cases typically demonstrate localized structural abnormalities on both sides; advanced disease is characterized by biventricular dilation and systolic impairment. The clinical picture is generally characterized by a composite of right-dominant and left-dominant features. Ventricular arrhythmias of both RBBB and LBBB configuration may occur, and at least 15% of cases show both morphologies of extrasystoles, underlining the presence of arrhythmogenic substrate in both ventricles. The ratio of RV to LV volume remains close to 1 throughout the disease course^[6].

Finally, it must be remembered that, as noted above, during the progression of the disease an initial right or left-dominant pattern can evolve into a biventricular dysfunction^[13,31].

Biventricular arrhythmogenic cardiomyopathy can mimic clinically and at imaging examinations a dilated CMP and be diagnosed only by pathologic examination at necropsy or of the explanted heart^[32].

CRITERIA AND CHALLENGES IF DIAGNOSIS

As mentioned above, the clinical diagnosis of ARVC is

Table 1 Revised arrhythmogenic right ventricular cardiomyopathy diagnostic criteria (modified from Marcus *et al.*^[33])

	Major criteria	Minor criteria
RV systolic function and structure	By 2D echo: Regional RV akinesia, dyskinesia or aneurysm and one of the following (end diastole): PLAX RVOT ≥ 32 mm, PSAX RVOT ≥ 36 mm, Or fractional area change $\leq 33\%$ By MRI: Regional RV akinesia, dyskinesia or aneurysm or dyssynchronous RV contraction and 1 of the following: Ratio of RV end-diastolic volume to BSA ≥ 110 mL/m ² or ≥ 100 mL/m ² (or RV EF $\leq 40\%$) By RV angiography: Regional RV akinesia, dyskinesia or aneurysm	By 2D echo: Regional RV akinesia, dyskinesia or aneurysm and 1 of the following (end diastole): PLAX RVOT ≥ 29 to < 32 mm, PSAX RVOT ≥ 32 to < 36 mm, Or fractional area change $> 33\%$ to $\leq 40\%$ By MRI: Regional RV akinesia, dyskinesia or aneurysm or dyssynchronous RV contraction and 1 of the following: Ratio of RV end-diastolic volume to BSA ≥ 100 to < 110 mL/m ² (male) or ≥ 90 to < 100 mL/m ² (female) or RV $> 40\%$ to $\leq 45\%$ By RV angiography: Regional RV akinesia, dyskinesia or aneurysm
Tissue characterization	Residual myocytes $< 60\%$ by morphometric analysis with fibrous replacement of the RV free wall myocardium in ≥ 1 sample, with or without fatty replacement of tissue on EMB	Residual myocytes 60% to 75% (or 50% to 65% if estimated), with fibrous replacement of the RV free wall myocardium in ≥ 1 sample, with or without fatty replacement of tissue on EMB
Repolarization abnormality	Inverted T waves in right precordial leads (V1-3) or beyond in individuals > 14 yr of age (in the absence of complete right bundle - branch block QRS ≥ 120 ms)	Inverted T waves in leads V1 and V2 in individuals > 14 years of age (in the absence of complete right bundle branch block) or in V4-6 or inverted T waves in leads V1-V4 individuals > 14 yr of age in the presence of complete right bundle branch block
Depolarization abnormality	Epsilon waves in the right precordial leads (V1-3)	Late potential by SAECC in ≥ 1 of 3 parameters in the absence of a QRS duration of ≥ 110 ms on the standard ECG; Filtered QRS duration ≥ 114 ms; Duration of terminal QRS < 40 mV or ≥ 38 μ s; Root-mean-square voltage of terminal 40 ms ≤ 20 μ V; Terminal activation duration of QRS ≥ 55 ms measured from the nadir of the S wave to the end of QRS
Arrhythmias	Nonsustained or sustained ventricular tachycardia of left bundle branch morphology with superior axis Frequent ventricular extrasystoles (> 1000 per 24 h) (Holter)	Nonsustained or sustained ventricular tachycardia of RV outflow configuration, left bundle branch morphology with inferior axis or > 500 ventricular extrasystoles per 24 h (Holter)
Familial history	ARVC confirmed pathologically in the first degree or identification of a pathogenic mutation categorized as associated or probably associated with ARVC	History of ARVC in a first degree relative or premature sudden death (< 35 yr of age) due to suspected ARVC or ARVC confirmed pathologically or by current Task Force Criteria in second-degree relative

LV: Left ventricle; RV: Right ventricular; ARVC: Arrhythmogenic right ventricular cardiomyopathy; PLAX: Parasternal long axis; PSAX: Parasternal short axis; RVOT: Right ventricle outflow tract; ECG: Electrocardiogram; EMB: Endomyocardial biopsy; MRI: Magnetic resonance imaging; SAECC: Signal averaged ECG; BSA: body surface area.

often difficult because of the non-specific nature of the disease and the broad spectrum of phenotypic expressions. Consequently, ARVC is probably underestimated as milder cases frequently go unrecognized and non-classic subtypes are not incorporated. Furthermore, left-dominant and biventricular arrhythmogenic CMP are commonly misattributed to dilated CMP^[32], hot phases to isolated viral myocarditis, and early disease to idiopathic ventricular tachycardia or benign ventricular ectopy^[5,6]. The common thought that ARVC is a disease of the young and cannot present beyond middle age is probably an erroneous assumption, which becomes self-fulfilling as clinicians fail to consider it as a possibility in older patients. Raising clinicians' awareness of the disease and its multiple presentations is critical to timely diagnosis and prevention of SCD.

There is no single gold-standard diagnostic test for ARVC, and the diagnosis relies on a scoring system with "major" and "minor" criteria based on the demonstration of a combination of defects in RV morphology and

function, characteristic depolarization/repolarization ECG abnormalities, characteristic tissue pathology, typical arrhythmias, family history, and the results of genetic testing^[33]. Definitive diagnosis, based on the Revised 2010 Task Force Criteria^[33] (Table 1), requires 2 major criteria, 1 major and 2 minor criteria, or 4 minor criteria from different categories. Therefore, the initial evaluation of all patients suspected of having ARVC should include physical examination, clinical history, family history of arrhythmias or SCD, ECG (Figures 3 and 4), signal-averaged ECG, Holter ECG monitoring, and comprehensive noninvasive imaging tests focused on both ventricles, such as echocardiography (Figure 5). New tools for improving diagnostic accuracy have been introduced in the clinical practice. Among non-invasive investigations, cardiac magnetic resonance (CMR) gives accurate morpho-functional evaluation of both ventricles with quantitative assessment of ventricular volumes and ejection fractions, and can give information on myocardial tissue characterization (fatty infiltration, and

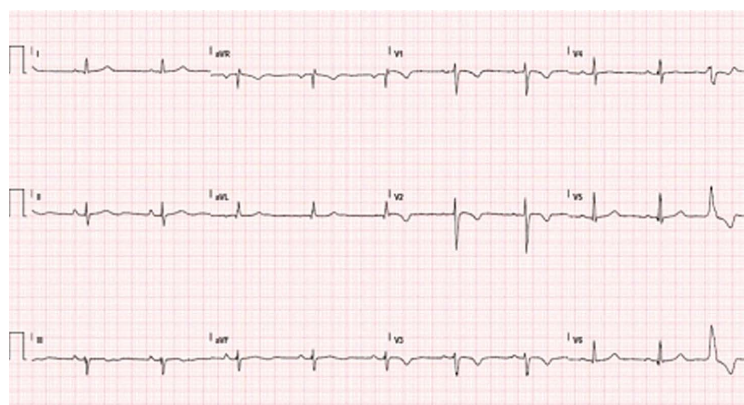


Figure 3 Typical electrocardiogram in a patient with classical arrhythmogenic right ventricular cardiomyopathy. Negative T waves in anterior precordial leads are present. A ventricular premature beat with left bundle branch block morphology is also observed.

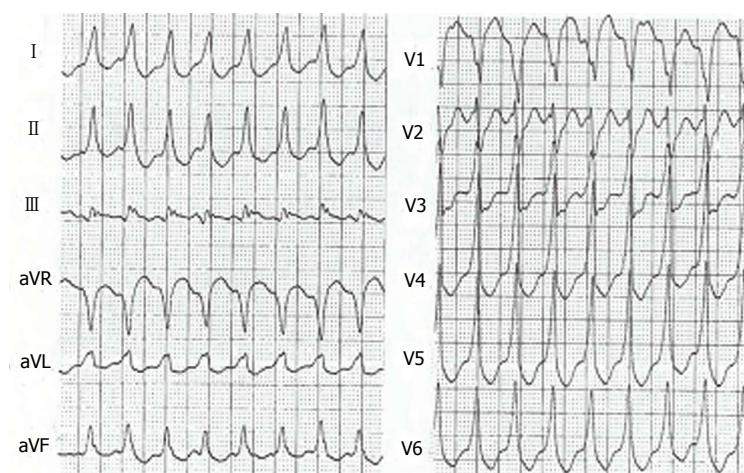


Figure 4 Ventricular tachycardia with left bundle branch block pattern in a patient with arrhythmogenic right ventricular cardiomyopathy.

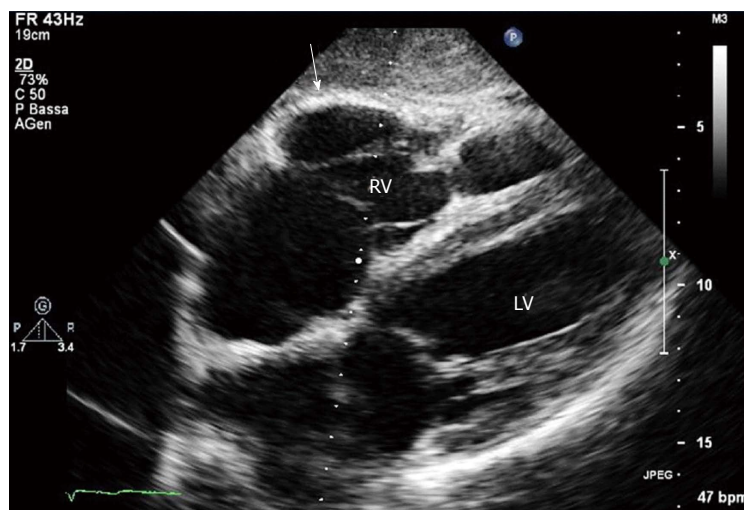


Figure 5 Two-dimensional echocardiogram, 4 chamber subcostal view, end diastolic frame, in an arrhythmogenic right ventricular cardiomyopathy patient. RV wall aneurysm at subtricuspid basal level is evident (arrow). LV: Left ventricle; RV: Right ventricle.

fibrosis, at LGE study) (Figure 6)^[34,35].

In the new ARVC guidelines^[33], “major” diagnostic criteria were selected because of their good sensitivity and specificity for the disease. It is important to note that imaging qualitative typical abnormalities of the disease, as aneurismal RV bulges are diagnostic only if associated with quantitative data as RV enlargement and/or depressed systolic function. Non-invasive tissue characterization by CMR was not considered because its poor specificity and reproducibility. Emerging major criteria is the demonstration of a typical genetic mutation, and genetic study is clinically

useful particularly in borderline or possible ARVC^[3-6]. If a noninvasive workup is suggestive but non diagnostic, further testing should be considered to establish the diagnosis, including electrophysiologic testing, RV angiography, electroanatomic mapping^[36], and rarely also endomyocardial biopsy. This invasive procedure was frequently employed in the past and has been considered the “gold-standard”, but presently it is indicated only in very selected cases, with questionable diagnosis of ARVC despite thorough diagnostic assessment^[6]. In fact, its sensitivity is not absolute, due to the frequent patchy distribution of the disease, and

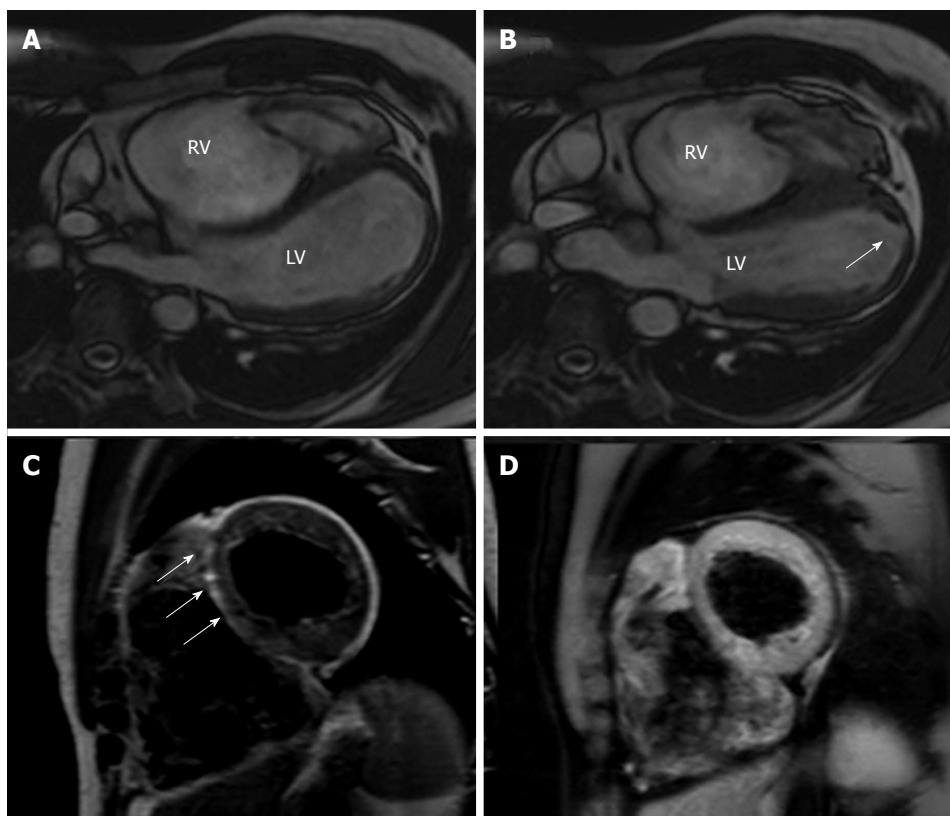


Figure 6 Cardiac magnetic resonance images of an arrhythmogenic right ventricular cardiomyopathy patient with mild left ventricular involvement. A, B: End-diastolic and end-systolic frames, off-axis 4 chamber view. Note the multiple bulging segments at right ventricular (RV) free wall. An apical hypokinesia of the left ventricle (LV) is also seen (arrow); C, D: Short axis T1-weighted dark blood imaging without (C) and with fat saturation (D) in the same patient with arrhythmogenic right ventricular cardiomyopathy. Note the hyperintense area in the interventricular (IV) septum in T1-weighted image (arrows) and corresponding hypointensity at T1-weighted dark blood imaging with fat-saturation indicating fatty infiltration in the IV septum.

the procedure is not without serious risk of RV perforation because of the abnormally thin RV wall characteristic of the disease. If a biopsy is scheduled, it must be analyzed by optimal technique using quantitative morphometry^[37], and the site of right ventricular puncture must be preferably chosen with echocardiographic, CMR or electroanatomic guidance^[3]. Moreover, recent data suggest the diagnostic usefulness of immunohistochemical analysis of plakoglobin signal level at intercalated discs, diffusely reduced in myocardial tissue of ARVC^[38].

DIFFERENTIAL DIAGNOSIS

Diagnosis of ARVC should be considered in any patient without a definite heart disease who presents with syncopal episodes, frequent ventricular extrasystoles or ventricular tachycardia. The main differential diagnoses include the following conditions:

Idiopathic RV outflow tract-ventricular tachycardia is a mostly benign condition not associated with structural heart disease. In early stage ARVC can be difficult to distinguish from this “idiopathic” type of ventricular arrhythmia in absence of structural changes^[4]. A scoring system has been developed to identify a concealed ARVC in patients with apparently idiopathic VT^[39]. Differential diagnosis is based on the fact that this arrhythmia is non-familial, and patients do not have the characteristic ECG/

signal average ECG abnormalities of ARVC (inverted T waves in V1-V3, epsilon waves, QRS duration > 110 ms). Accurate imaging examination with CMR, and systematic follow-up reassessment can be useful to exclude RV abnormalities.

Brugada syndrome is an inherited cardiac condition that, similarly to ARVC, can be transmitted with an autosomal dominant pattern, and can lead to SCD from malignant ventricular arrhythmias. Differently from ARVC, it is characterized by a distinct typical ECG pattern with “J wave” in precordial leads, and absence of RV morpho-functional abnormalities at imaging.

Dilated cardiomyopathy may be difficult to distinguish from ARVC, especially in its advanced stage with severe biventricular involvement. In absence of classic ARVC hallmarks (RV aneurysms, bulging), the clinical distinction between these 2 CMP can be very difficult or impossible^[32].

Myocarditis can mimic ARVC, especially when the RV is involved. Myocarditis can cause structural abnormalities, including microaneurysms, as well as the arrhythmic manifestations considered typical of ARVC. Moreover, myocardial inflammatory infiltrates, myocyte necrosis, replacement fibrosis and also fibro-fatty replacement of the RV myocardium can be observed also in myocarditis, resembling ARVC histologic features. New tools, such as 3-dimensional electro-anatomic mapping, applied to the standard endomyocardial biopsy, have been introduced to

improve diagnostic accuracy in the clinical practice. Recently, in a provocative study Pieroni *et al.*^[40], found that 50% of patients with a noninvasive ARVC diagnosis fulfilled Dallas histological criteria of active myocarditis. These data would require confirmation in the future on large patient populations.

Sarcoidosis with cardiac involvement can mimic ARVC, making an accurate differential diagnosis is particularly challenging^[6]. Cardiac sarcoidosis must be suspected in presence of concomitant mediastinal lymphadenopathy, extracardiac sarcoidosis, conduction defects with a high-grade atrio-ventricular block, and interventricular septal scar at imaging. A global RV hypokinesis or regional wall motion abnormalities can be present, due to the patchy nature of the granulomatous infiltration. The absence of myocardial fat infiltrates at CMR could be useful distinguishing feature to suspect cardiac sarcoidosis^[41], although its diagnostic accuracy could vary, depending on the stage of the disease at which the CMR data were acquired. Endomyocardial biopsy may be indicated in selected cases with questionable diagnosis.

Other pathologies: (1) coronary artery disease and myocardial infarction can involve both ventricles and mimic aspects of ARVC; (2) pulmonary hypertension (RV pressure overload), and/or significant tricuspid regurgitation (RV volume overload) can cause RV dilation and dysfunction; (3) congenital heart diseases such as Uhl's anomaly (a rare congenital heart disease with a total loss of the RV myocardial muscle and parchment appearance)^[42] and repaired Tetralogy of Fallot have to be consider especially for their prevalent RV involvement; and (4) intracardiac left to-right shunts (*e.g.*, atrial septal defects and anomalous pulmonary venous drainage) may cause RV volume overload. The diagnosis can be missed on standard echocardiogram, and transesophageal echocardiography and/or CMR can improve the diagnostic accuracy, in these selected cases.

PATIENT MANAGEMENT

Prevention of SCD is the most important management task for the patients affected by ARVC. Retrospective analysis of clinical and pathological series identified several risk factors, such as previous cardiac arrest, syncope, young age, malignant family history, participation in competitive sports, ventricular tachycardia, severe RV dysfunction, LV involvement, and QRS dispersion^[43,44]. However, it has to be noted that the prognostic value of these single or combined risk factors has not been prospectively assessed. Two recent papers^[45,46] tried to define the incidence and predictors of ICD therapy in patients with ARVC after placement of an ICD for primary prevention. Nearly one-half of the ARVC patients with primary prevention ICD implantation experienced appropriate ICD interventions. In one or both of these studies, proband status of patients, presence of unexplained syncopal episodes, inducibility of ventricular arrhythmias at electrophysiologic study and presence of non-sustained ventricular tachycardia at Holter monitoring resulted independent predictors of appropriate

ICD discharge.

In summary, according to the International Guidelines and the consensus of the experts^[47,48], the indications of ICD for prevention of SCD in ARVC patients are well established for high-risk patients with history of aborted SCD or episodes of sustained ventricular tachycardia (Level of Recommendation: IB), while in presence of unexplained syncope, non-sustained ventricular tachycardia, familial history of sudden death, extensive disease including those with LV involvement and are considered as possible indications for ICD at intermediate risk of SCD (Class of Recommendation II a, Level of evidence: C). Additionally, the rare patients with genotypes of ARVC associated with a high genetic risk for SCD (*e.g.*, ARVC 5)^[49] may be considered as possible candidates for ICD therapy. It is currently recommended that asymptomatic patients have to be managed in a case-by-case basis.

The role of the electrophysiology with programmed ventricular stimulation remains controversial in the specific setting of ARVC. In fact, contrary to the above mentioned study by Bhonsale *et al.*^[45], in the Darwin II study^[46], this test showed poor accuracy in predicting appropriate ICD interventions.

The impact of ICD implantation in ARVC has been evaluated in a recent meta-analysis^[50]: a total of 610 patients were collected from 18 cohorts with ICD for either primary or secondary prevention and the annualized rate of appropriate ICD therapies resulted 9.5%.

The clinical relevance of ICD implantation in improving survival in patients with ARVC was clearly demonstrated by Corrado *et al.*^[46], by comparing the actual survival curve of their implanted patients with the ventricular fibrillation/flutter-free survival (estimated mortality reduction at 48 mo of 23%).

In ARVC patients, pharmacologic treatment as well as radiofrequency ablation (RFA) must not be considered a definitive therapy for ventricular arrhythmias, and they are not an equivalent alternative to ICD therapy in patients at high risk of SCD. RFA can be appropriate in selected patients who are not candidates for an ICD, or in those with an ICD who have frequent episodes of VT and ICD shocks despite antiarrhythmic drugs. Multiple recent studies suggest that simultaneous epicardial and endocardial approaches for VT mapping and ablation are feasible - although technically more demanding - and might even result in suppression of recurrent VT. This could be explained by the preferential epicardial infiltration characteristic of the disease^[51].

Antiarrhythmic medications have been used for symptomatic control in ARVC. The combination of beta-blockers and amiodarone has a proved beneficial effect in suppression of non-sustained VT, in reduction of sustained VT arrhythmias and rate, preventing syncope and favoring anti-tachycardia pacing termination rather than shock therapy. Hence, sotalol and amiodarone have been proposed as effective treatment of sustained VT or VF as adjunctive therapy to ICD or in patients with ARVC that are not candidates for ICD implantation (Class of Recommendation II a, Level of evidence: C)^[48,52].

Furthermore, the North American ARVC Registry has demonstrated that amiodarone alone showed greatest efficacy at preventing sustained ventricular tachycardia or ICD discharge^[53]. Conversely, a study from our group^[54] reported that the treatment with amiodarone was independently related with increased mortality, presumably because the treated cases were those with higher arrhythmic risk.

Beta-blockers and angiotensin-converting enzyme inhibitors can be also used in ARVC patients, particularly in those with biventricular dysfunction and HF, due to their proven benefit in reducing mortality and slowing disease progression in other CMP, although no studies are presently available specifically on the response of ARVC patients to these medications^[6].

General life education measures are also important in ARVC patients. Particular caution must be addressed to avoid competitive sport activities and strong physical efforts^[6], which could increase the phenotypic expression and the arrhythmic risk^[55,56].

Cardiac transplantation is indicated in patients with severe intractable heart failure, generally with end-stage disease and severe biventricular involvement, and in selected cases with intractable incessant ventricular arrhythmias^[57].

UNSOLVED PROBLEMS AND FUTURE PERSPECTIVES

Despite considerable improvement in knowledge, ARVC has still several unsolved problems that deserve further research. In our opinion, the main future challenges would answer to the following questions: (1) what is the clinical role of genetic testing^[6,17,58,59]? (2) how to improve the identification of affected cases, particularly in the concealed phase and in disease variants (LDAC, atypical forms)? and (3) how to improve the risk stratification of patients?

The use of genetic testing is growing very rapidly in recent years in CMPs^[17], and its role is changing from a research tool to a clinically useful exam. In our opinion, based on the present knowledge, its clinical role in ARVC is not well defined. In fact, a pathogenic mutation can be recognized only in approximately a half of probands, and the possibility of multiple mutations or a non pathogenic benign mutation, encountered also in healthy individuals can cause considerable diagnostic problems^[59-61]. A genetic study is considered clinically useful in equivocal cases, because the demonstration of a pathogenic mutation is a major criteria in the revised ARVC diagnostic criteria^[33], and with a “cascade” analysis, in relatives of ARVC patients with identified mutation^[17-60]. Appropriate genetic counseling and clinical management are very important particularly in genetic positive apparently healthy familial subjects (regular follow-up visits, possible caution about competitive sports). A clinically oriented approach which considers the presence of diagnostic “red flags” is preferable, in order to help in the proper selection of candidate genetic mutations^[58].

New methods for early and precise identification of

ARVC in initial phases are presently under active research. Advanced echocardiographic analyses can be helpful, particularly the study of myocardial deformation using speckle tracking analysis^[62-64]. The modification of diagnostic ARVC criteria by the recent revision^[33] significantly improved the diagnostic power of available methods, increasing both sensitivity and specificity^[20,62,65]. However it has to be observed that the diagnostic criteria of LDAC were not considered in the last task force revision, and that would be advisable in the near future^[7].

Accurate risk stratification is problematic in patients with ARVC, particularly for patients without history of severe life threatening arrhythmias (primary prevention of SCD)^[44].

Additional potentially useful prognostic data recently were demonstrated by cardiovascular imaging, such as echocardiography^[54,66], CMR^[67,68], and electroanatomic mapping^[69], thus reinforcing the importance of identifying the pathologic substrate of arrhythmias in the disease (areas of myocardial scarring and fibro-fatty infiltration with a probable reentry mechanism). Bhonsale *et al*^[70] recently proposed a strategy for risk stratification for ARVC associated desmosomal mutation carriers based on pedigree evaluation, ECG and Holter information.

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Experimental models of inherited cardiomyopathy and its therapeutics

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Abstract

Cardiomyopathy is a disease of myocardium categorized into three major forms, hypertrophic (HCM), dilated (DCM) and restrictive cardiomyopathy (RCM), which has recently been demonstrated to be a monogenic disease due to mutations in various proteins expressed in cardiomyocytes. Mutations in HCM and RCM typically increase the myofilament sensitivity to cytoplasmic Ca^{2+} , leading to systolic hyperfunction and diastolic dysfunction. In contrast, mutations in DCM typically decrease the myofilament sensitivity to cytoplasmic Ca^{2+} and/or force generation/transmission, leading to systolic dysfunction. Creation of genetically-manipulated transgenic and knock-in animals expressing mutant proteins exogenously and endogenously, respectively, in their hearts provides valuable animal models to discover the molecular and cellular mechanisms for pathogenesis and promising therapeutic strategy *in vivo*. Recently, cardiomyocytes have been differentiated from patient's induced pluripotent stem cells as a model of inherited cardiomyopathies *in vitro*. In this review, we provide overview of experimental models of cardiomyopathies

with a focus on revealed molecular and cellular pathogenic mechanisms and potential therapeutics.

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Key words: Cardiomyopathy; Gene; Mutation; Animal model; Induced pluripotent stem cell; Therapeutics

Core tip: Current experimental models of inherited cardiomyopathies (hypertrophic cardiomyopathy, dilated cardiomyopathy and restrictive cardiomyopathy), including genetically-manipulated mouse models (transgenic and knock-in mice) and patient's induced pluripotent stem cell-derived cardiomyocyte models, are summarized and discussed with a focus on revealed molecular pathogenic mechanisms and potential drug therapeutics.

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INTRODUCTION

Cardiomyopathies are categorized, based on ventricular morphology and function, into three major forms, hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM)^[1]. HCM is characterized by increased left ventricular (LV) wall thickness, cardiomyocyte disarray, increased myocardial fibrosis and impaired LV diastolic function with normal or increased LV systolic function^[2-4]. DCM is characterized by LV dilatation and systolic dysfunction, frequently resulting in heart failure, arrhythmias and sudden death, with heart transplantation being the most effective treatment for survival at end stage because of no effective therapeutic drugs^[5]. RCM is an uncommon form of cardiomyopathy, characterized by restrictive filling of LV and/or right ventricle despite normal

or near-normal wall thickness and systolic function^[6,7].

Following the uncovering of a gene mutation in β -myosin heavy chain (β -MyHC) of familial HCM patients at 1990^[8], a large number of mutations in the genes encoding sarcomere proteins in cardiac muscle have been found to cause HCM, DCM and RCM^[9]. Many animal models have been created to discover the functional consequences of these mutations and molecular mechanisms for the pathogenesis of cardiomyopathies *in vivo*, which should be critical for advancement of diagnosis and therapy. Recently, premature cardiomyocytes have been created from induced pluripotent stem cells (iPSC) of patients with inherited cardiomyopathies as a novel disease model *in vitro*. This review summarizes the recent advances in our understanding about molecular pathogenic mechanisms and potential therapeutic strategy brought about from these experimental models.

HYPERTROPHIC CARDIOMYOPATHY

HCM, characterized by unexplained LV wall thickening and diastolic dysfunction, has an overall prevalence of 200 per 100000 individuals^[10]. It is known that LV systolic function is not impaired but rather increased in HCM patients^[2]. Structural remodeling involving hypertrophic growth of LV is believed to be caused by enhanced protein synthesis in cardiomyocytes leading to hyperplasia of myofibrils and thus cardiomyocyte enlargement. The purpose of current therapy for HCM is to improve diastolic dysfunction indirectly through suppressing systolic function using β -blockers, Ca^{2+} channel blockers or Na^+ channel blockers^[11-13].

Human HCM is a monogenic disorder, which is caused by several hundred distinct mutations in many genes found in patients and families with HCM^[14,15]. The causal genes for HCM include those encoding cardiac myosin-binding protein C (MYBPC3), β -MyHC (MYH7), cardiac troponin C (TNNT1), cardiac troponin I (TNNI3), cardiac troponin T (TNNT2), cardiac actin (ACTC), α -tropomyosin (TPM1), regulatory myosin light chain, essential myosin light chain and titin/connectin. Mutations in these genes account for approximately 65% of all HCM cases^[16], indicating that HCM is a disease of sarcomeric protein genes. The total number of mutations in each genes increase depending on the gene size, so that any one of mutations in two large genes encoding MYH7 and MYBPC3 are identified in about 50% of cases while mutations in other genes only account for less than 20% of cases^[16].

Soon after discovery of these mutations in sarcomeric proteins, extensive studies have been started to understand the pathogenic mechanisms by exploring the effects of mutations on the *in vitro* sarcomeric function as well as the *in vivo* global structure and function of the heart using genetically modified animal models. *In vitro* studies revealed that HCM-linked mutations in thin filament-associated regulatory proteins, including TNNT2, consistently increase the myofilament sensitivity to cytoplasmic Ca^{2+} and thus probably impair diastolic function through a malfunction in the troponin-tropomyosin regulatory system^[17-26]. Animal

models of human HCM with mutations in cardiac troponin T^[17,19,20,22,24], TNNI3^[21,23] and TPM1^[18,25,26] demonstrated that increased cardiac myofilament Ca^{2+} sensitivity is a root cause that initiates molecular cascades involving pathological cardiac remodeling in HCM. These findings indicate that reversal of the increased myofilament Ca^{2+} sensitivity toward normal levels is a promising definitive therapeutic strategy for HCM. At present, however, there exists no drugs that decrease the myofilament Ca^{2+} sensitivity through directly acting on the thin filament regulatory system, making it worthwhile to develop novel drugs “ Ca^{2+} desensitizers”. Epigallocatechin gallate, a major polyphenol in green tea, is a potential lead compound for Ca^{2+} desensitizers, which has been demonstrated to decrease the myofilament Ca^{2+} sensitivity in membrane-permeabilized cardiac muscle fibers through binding to a C-terminal lobe region of TNNT1^[27]. Poor absorption from the intestine and permeability into cells, however, may be serious problems to be solved. Another potential lead compound is blebbistatin, which has also been demonstrated to decrease the myofilament Ca^{2+} sensitivity in membrane-permeabilized cardiac muscle fibers through inhibiting the interaction between actin and myosin and prevent arrhythmia induced by Ca^{2+} sensitizer^[28]. Crossing transgenic mice harboring HCM-linked sarcomeric mutation with transgenic mice harboring DCM-linked sarcomeric mutation conferring decreased myofilament Ca^{2+} sensitivity was found to normalize overall myofilament Ca^{2+} sensitivity and prevent cardiac deterioration^[29,30], supporting the idea that Ca^{2+} desensitizer might be beneficial for HCM patients affected by mutations in sarcomeric protein genes.

HCM-causing mutations that increase the myofilament sensitivity to cytoplasmic Ca^{2+} also alter the regulation of intracellular Ca^{2+} level, which could activate hypertrophic response and failure in the myocardium^[31]. Cardiomyocytes isolated from experimental mouse models of HCM show abnormal intracellular Ca^{2+} handling, including increased diastolic Ca^{2+} associated with decreased Ca^{2+} store in the sarcoplasmic reticulum (SR), and dysregulation of intracellular Ca^{2+} precede hypertrophic remodeling of the heart^[32,33]. The voltage-dependent L-type Ca^{2+} channel inhibitor, diltiazem, restored the normal intracellular Ca^{2+} handling and suppressed cardiac hypertrophy in young mice with HCM-causing myosin R403Q mutation^[33], indicating that pharmacologic interventions targeting early key intracellular events caused by abnormal intracellular Ca^{2+} regulation could prevent disease development.

DILATED CARDIOMYOPATHY

DCM is characterized by progressive LV dilatation and systolic dysfunction, being the most common indication for cardiac transplantation^[5]. Many mutations in various genes encoding sarcomeric proteins, cytoskeletal proteins, nuclear envelope proteins and sarcolemmal membrane proteins have been shown to be linked to approximately 25%-30% of the DCM cases^[34-39]. Cardiomyocyte hypertrophy and fibrosis, but not cardiomyocyte disarray, are commonly observed as in the case of HCM^[36]. DCM is frequently accompanying with abnormal cardiac conduction system, arrhythmias

and sudden death probably due to pathophysiological myocardial remodeling and severe fibrosis. Underlying molecular mechanisms include diminished force generation/transmission, altered energy metabolism, and impaired intracellular calcium handling in cardiomyocytes^[3]. The purpose of current standard therapy for DCM is to prevent the progression of myocardial remodeling and systolic dysfunction by a combination of cardioprotective drugs, including β -adrenergic receptor blockers, vasodilators (angiotensin converting enzyme inhibitors or angiotensin II receptor blockers), aldosterone antagonists and diuretics^[40].

In contrast to HCM-causing mutations, DCM-causing mutations in TPM1^[41] and TNNT2 consistently decrease the myofilament sensitivity to cytoplasmic Ca^{2+} and thus impair systolic function through a malfunction in the troponin-tropomyosin regulatory system^[42,43]. A mouse model of DCM caused by the deletion mutation ΔK210 in TNNT2 demonstrated that lessened cardiac myofilament Ca^{2+} sensitivity is a root cause that initiates molecular cascades involving pathological cardiac remodeling in DCM^[44]. This mouse model developed an early-onset severe LV dilation with high incidence of sudden death despite showing no heart failure symptoms, resembling the phenotypes of a human family of DCM patients with this mutation^[35]. These findings indicate that reversal of the decreased myofilament Ca^{2+} sensitivity toward normal levels is a promising definitive therapeutic strategy for DCM linked to sarcomeric regulatory protein gene mutations. Early intervention with a Ca^{2+} sensitizer, pimobendan, had remarkable effects of preventing cardiac remodeling, systolic dysfunction and sudden death in this DCM model mouse^[44]. However, it remains to be determined whether pimobendan has also therapeutic effects on DCM mice with this mutation after developing decompensated, end-stage heart failure. It may be worth noting that combination therapy with pimobendan and β -blocker has provided beneficial effects in DCM patients with severe heart failure^[45,46].

Cardiomyocyte contraction is evoked by Ca^{2+} , which is rapidly released into cytoplasm from SR upon sarcolemmal depolarization. Cytoplasmic Ca^{2+} is rapidly returned to a low level during diastole by reuptake into SR through SR Ca^{2+} pump (SERCA2a). Myocardial expression of *SERCA2a* is down-regulated in the patients with end-stage congestive heart failure^[47,48], resulting in a decrease in the rate of Ca^{2+} reuptake by SR^[49-51]. Myocardial expression of *SERCA2a* was also confirmed to be markedly decreased in a mouse model of DCM^[52]. In a pressure-overload heart failure model of rats, transfection of adenovirus expression vector carrying *SERCA2a* cDNA into the heart normalized the hemodynamic parameters, including LV end-systolic pressure, maximum rates of LV pressure increase and decrease, and isovolumic relaxation rate^[53]. Another study using a pressure-overload model of rats demonstrated that adenoviral transfection of *SERCA2a* during heart failure reversed the LV dilation and improved the myocardial energy metabolism and survival^[54]. *SERCA2a* gene transfer also improved the contractile function of cardiomyocytes taken from patients with heart failure by increasing the rates of contraction and

relaxation, decreasing and increasing the cytoplasmic Ca^{2+} at diastole and systole, respectively, and normalizing the frequency dependence of force generation^[55]. Taken together, these studies suggest that enhancement of *SERCA2a* expression in cardiomyocytes may serve as potential therapeutic strategy for DCM patients.

RESTRICTIVE CARDIOMYOPATHY

RCM is characterized by increased stiffness of ventricular chambers, with wall thickness and systolic function usually being within normal limits. The reduction in myocardial compliance results in an abnormally large increase in early diastolic ventricular pressure against small increment in volume and an abrupt termination of filling. Most individuals with RCM develop heart failure and die within a few years^[56]. Several reports suggest clinical and genetic overlaps between RCM and HCM^[56-58]. RCM is rare, and its genetic etiology has just started to be explored. To date, RCM-linked mutations are found in sarcomere protein genes, including *TNNI3*, *TNNT2*, *MYH7* and *ACTC*^[58-61].

Like sarcomeric gene mutations in other types of cardiomyopathy, RCM-causing sarcomeric gene mutations alter myofilament sensitivity to cytoplasmic Ca^{2+} through a malfunction in the troponin-tropomyosin regulatory system. Membrane-permeabilized cardiac muscle fibers prepared from transgenic mouse model of RCM are more sensitive to Ca^{2+} and show more force at low Ca^{2+} levels than those from transgenic mice overexpressing wild-type proteins^[62]. This is consistent with the findings from earlier *in vitro* studies in which recombinant RCM-causing mutant proteins are exchanged into membrane-permeabilized cardiac muscle fibers^[63-65]. Kobayashi *et al.*^[66] demonstrated that the increase in myofilament Ca^{2+} sensitivity was caused by increased affinity of troponin C for Ca^{2+} in the thin filament. Thus, the myofilament hypersensitivity to cytoplasmic Ca^{2+} is a common feature that RCM-causing mutations share with HCM-causing mutations. *In vitro* experiments using membrane-permeabilized cardiac muscle fibers reconstituted with recombinant mutant proteins revealed that RCM-causing mutations give much greater Ca^{2+} sensitivity to the myofilament compared with HCM-causing mutations^[62,63]. Consistent with these *in vitro* reconstitution experiments, membrane-permeabilized cardiac muscle fibers prepared from transgenic mice expressing RCM-causing TNNI3 R145W mutant showed a much larger increase in the Ca^{2+} sensitivity of ATPase activity and force generation compared with those from transgenic mice expressing HCM-causing TNNI3 R145G mutant^[62,67]. Crossing transgenic mice expressing RCM-causing TNNI3 R193H mutant with transgenic mice expressing N-terminal truncated TNNI3, known to decrease myofilament Ca^{2+} sensitivity, corrected the impaired relaxation in R193H RCM transgenic mice^[68], supporting the idea that myofilament Ca^{2+} desensitizer could also be beneficial to treat RCM caused by sarcomeric protein gene mutations. Design of new compounds that exert lusitropic action on the heart directly through decreasing the myofilament Ca^{2+} sensitivity is an innovative and exciting

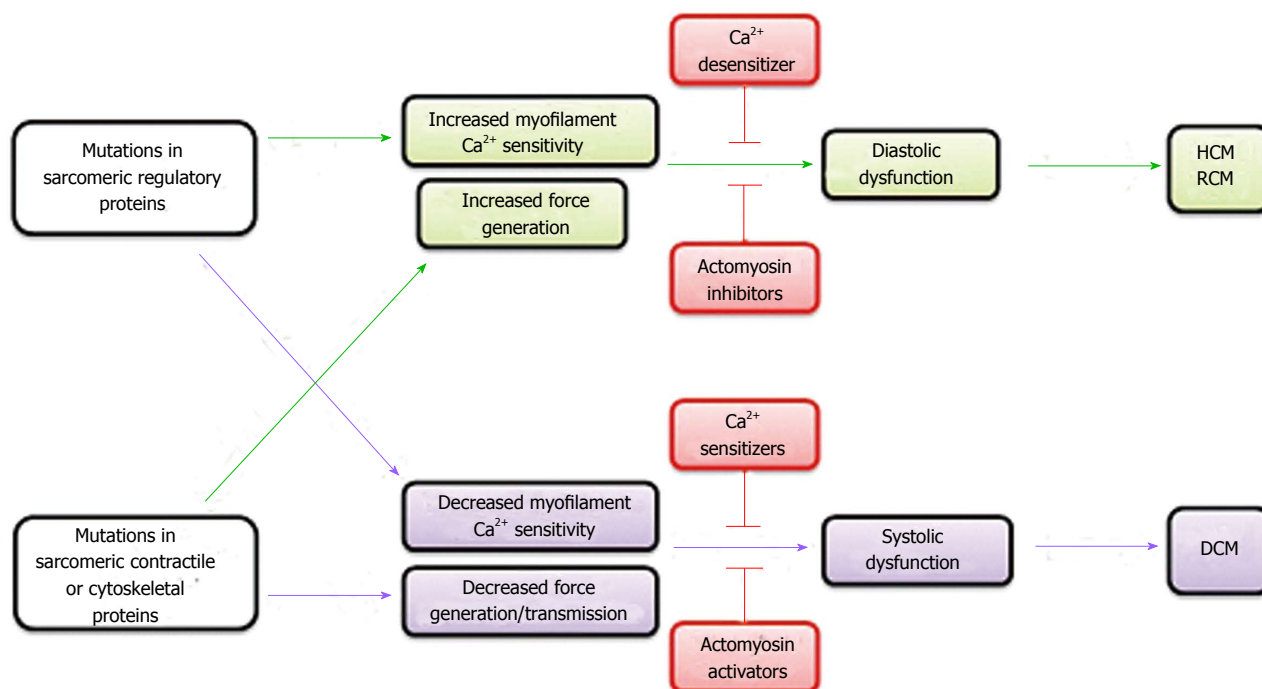


Figure 1 Essentials of pathogenic mechanisms in inherited cardiomyopathies and potential definitive drug therapies. HCM: Hypertrophic cardiomyopathy; DCM: Dilated cardiomyopathy; RCM: Restrictive cardiomyopathy.

challenge to overcome RCM as well as HCM.

CARDIOMYOCYTES DIFFERENTIATED FROM PATIENT'S INDUCED PLURIPOTENT STEM CELLS AS AN *IN VITRO* MODEL FOR INHERITED CARDIOMYOPATHIES

Although the contribution of gene-manipulated animal models to the understanding of inherited cardiomyopathies in *in vivo* system has been enormous, small animals have significantly different intrinsic properties in the heart from human, including faster heart rate, shorter plateau phase in the action potential of ventricles, and much higher ratio of α/β -MyHC isoforms in ventricles. Intact cardiomyocytes are difficult to obtain from healthy person and even from cardiomyopathy patients. The iPSC technology may offer a unique opportunity for creating disease-specific models directly from human patients with monogenic disease to investigate underlying mechanisms and carry out drug screening in human cardiomyocytes, though only *in vitro*^[69,70]. Premature but self-beating cells like cardiomyocytes have been shown to be differentiated from human iPSC^[71,72]. Patient-specific iPSC-derived cardiomyocytes have been created for HCM-causing missense mutation R663H in MYH7^[73]. These iPSC-derived cardiomyocytes developed cellular hypertrophy and arrhythmia at the single cell level accompanying irregular Ca^{2+} cycling and elevation in resting cytoplasmic Ca^{2+} level. Further, pharmacological inhibition of Ca^{2+} entry with L-type Ca^{2+} channel blockers verapamil, nifedipine and diltiazem prevented development of cellular hypertrophy and electrophysiological abnormality.

It is somewhat surprising that these numerous aspects of HCM phenotype can be reproduced in an *in vitro* cultured system without any neurohormonal stimulation, since these phenotypes are thought to develop as a long-term consequence of adaptation or compensation *in vivo* to an abnormal contractile function conferred by the mutation in a motor protein encoded in MYH7. The results of this study on patient-specific iPSC-derived cardiomyocytes, however, clearly show that iPSC-derived cardiomyocytes are a useful platform to elucidate molecular and cellular pathogenic mechanisms underlying inherited HCM and to identify novel therapies for this disease.

iPSC-derived cardiomyocytes from a three-generation family of DCM patients affected by a missense mutation R173W in TNNT2 have been shown to exhibit a lessened force generation capability, one of the common root causes for DCM, with impaired Ca^{2+} handling and abnormal distribution of Z-band α -actinin but no abnormalities in electrophysiological properties and cell size^[74]. β 1-selective adrenergic receptor blocker metoprolol improved the sarcomeric disorganization judged by α -actinin distribution, and over-expression of SERCA2a improved contractile function and Ca^{2+} handling. These findings demonstrated that cardiomyocytes differentiated from iPSCs of DCM patients recapitulated the disease phenotype to some extent and could be used as an *in vitro* experimental model to explore molecular and cellular pathogenic mechanisms underlying inherited DCM and to carry out drug screening for this disease.

CONCLUSION

Abnormal sensitivity to cytoplasmic Ca^{2+} or force generation/transmission of cardiac myofilament, which is

incurred as a direct functional consequence of mutations in genes encoding proteins in cardiomyocytes, is the primary root cause that initiates subsequent molecular and cellular events leading to pathological remodeling in inherited cardiomyopathies. HCM/RCM-causing mutations usually heighten the myofilament sensitivity to cytoplasmic Ca^{2+} or force generation, whereas DCM-causing mutations lessen the myofilament sensitivity to cytoplasmic Ca^{2+} or force generation/transmission. Therefore, reversal of the altered myofilament Ca^{2+} sensitivity or force generation/transmission capability toward normal levels should be a promising definitive therapeutic strategy to prevent or even reverse the progression of the disease in inherited cardiomyopathies (Figure 1). Further studies using gene-manipulated animal models and patient's iPSC-derived cardiomyocytes briefly summarized in this review are important to develop novel therapeutic drugs for inherited cardiomyopathy patients.

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Arginine vasopressin as a target in the treatment of acute heart failure

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Abstract

Congestive heart failure (CHF) is one of the most common reasons for hospitalization in the United States. Despite multiple different beneficial medications for the treatment of chronic CHF, there are no therapies with a demonstrated mortality benefit in the treatment of acute decompensated heart failure. In fact, studies of inotropes used in this setting have demonstrated more harm than good. Arginine vasopressin has been shown to be up regulated in CHF. When bound to the V1a and/or V2 receptors, vasopressin causes vasoconstriction, left ventricular remodeling and free water reabsorption. Recently, two drugs have been approved for use that antagonize these receptors. Studies thus far have indicated that these medications, while effective at aquaresis (free water removal), are safe and not associated with increased morbidity such as renal failure and arrhythmias. Both conivaptan and tolvaptan have been approved for the treatment of euvoletic and hypervolemic hyponatremia. We review the results of these studies in patients with heart failure.

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Key words: Heart failure; Arginine vasopressin antagonist;

Vaptan; Hyponatremia; Aquaresis; Vasopressin

Core tip: Beneficial therapies in the setting of acute decompensated heart failure are limited. When bound to the V1a and/or V2 receptors, vasopressin, which is upregulated in heart failure, causes vasoconstriction, left ventricular remodeling and free water reabsorption. Over recent years, vasopressin antagonists such as conivaptan and tolvaptan have been investigated and approved for use in the appropriate setting. We review the evidence and implications behind use of vaptans in the setting of heart failure.

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INTRODUCTION

Congestive heart failure (CHF) is a growing problem, with high mortality, frequent hospitalizations and poor quality of life. CHF afflicts about 5 million people in the United States, with over half a million new diagnoses and 200000 deaths each year^[1,2]. Despite advances in therapy such as the use of angiotensin converting enzyme (ACE) inhibitors and beta blockers, heart failure hospitalizations are on the rise, with over a million a year, partly due to patients living longer and surviving acute myocardial infarctions. One of the principle goals of therapy during a heart failure admission is to relieve excess volume in order to improve symptoms. This is primarily accomplished with the use of diuretics and vasodilators. Although these agents improve symptoms, they may be associated with an increase in mortality chronically^[1,3,4]. Additionally, they are often associated with hyponatremia^[5]. In an attempt to further advance heart failure treatment, several new medications have been studied

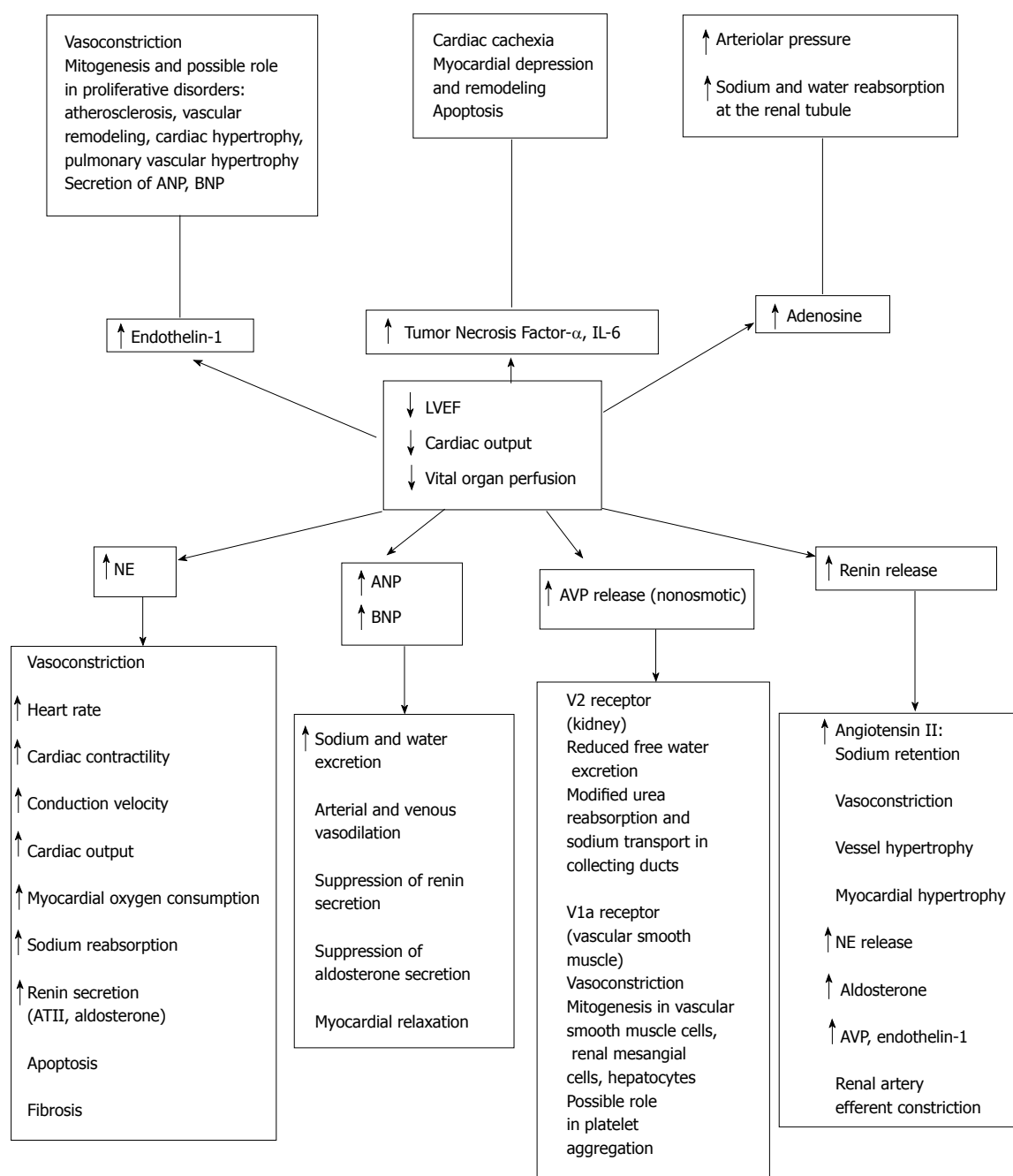


Figure 1 Summary of neurohormonal activation in heart failure. With injury to the left ventricle and subsequent decrease in left ventricular function, there is a decrease in cardiac output, subsequent decrease in perfusion of vital organs, and activation of the various neurohormonal systems. ANP: Atrial natriuretic peptide; AT II: Angiotensin II; AVP: Arginine vasopressin; BNP: Brain natriuretic peptide; IL-6: Interleukin 6; LVEF: Left ventricular ejection fraction; NE: Norepinephrine. From Russell *et al*^[28] with permission from Springer.

including natriuretic peptides, adenosine antagonists and vasopressin antagonists. The purpose of this paper is to review the role of vasopressin antagonists for the therapy of acute heart failure exacerbations.

NEUROHORMONAL ACTIVATION IN ACUTE HEART FAILURE

Acute heart failure is associated with activation of several components of the neurohormonal system. In response to ventricular dysfunction and decreased perfusion, baroreceptors in the aorta, carotid body, and the kidney are activated. The

immediate response is an increase in sympathetic nervous system outflow. Norepinephrine release results in tachycardia, arterial vasoconstriction, venoconstriction, and increased contractility. The renin-angiotensin-aldosterone system, which promotes the retention of sodium and subsequently water, is also activated. Additionally, arginine vasopressin (AVP), endothelin, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), adenosine, and tumor necrosis factor are all released. These hormones have a variety of individual effects as outlined in Figure 1.

Although the acute effects of these neurohormones are helpful to sustain life, chronically elevated levels may be

quite detrimental. In both the Studies of Left Ventricular Dysfunction (SOLVD) Trial and the Vasodilator - Heart Failure Trial II (V-HeFT II), investigators demonstrated that plasma levels of norepinephrine, renin, ANP, and AVP are elevated in patients with left ventricular dysfunction when compared with healthy controls^[6,7]. Furthermore, as New York Heart Association (NYHA) functional class worsens, the levels of these neurohormones are increased. Many of the beneficial effects of ACE inhibitors and beta blockers may be due to the blockade of these neurohormones. However, evidence for the use of these agents in reducing mortality is primarily in the chronic heart failure setting, and less is known about appropriate optimal management of acute heart failure.

Many studies have examined the effects of chronic diuretics on mortality in patients with heart failure. Cooper *et al*^[3] performed a retrospective analysis of the SOLVD Trial and found that those using a diuretic at baseline were more likely to have an arrhythmic death than those not on a diuretic. Even after controlling for disease severity, comorbidities, and concomitant medications, the use of diuretics was associated with an increased risk of arrhythmic death. Similar results were found in a retrospective analysis of 1153 patients from the Prospective Randomized Amlodipine Survival Evaluation Trial, which examined the use of amlodipine in patients with NYHA functional class IIIb/IV heart failure^[1]. High chronic doses of diuretics were associated with increased mortality, sudden death, and pump failure death. Although it is not surprising that higher diuretic doses are used in patients that have more advanced heart failure, a multivariate analysis controlling for disease severity revealed that high diuretic dose was still a predictor of mortality. This could possibly be explained by diuretic resistance, neurohormonal activation, or electrolyte changes rather than the dose itself. In fact, in the acute setting, higher-dose diuretic therapy has been shown to result in improved fluid loss and relief of congestive symptoms, lower adverse events, and despite acutely worsening renal function no difference in 60 d clinical outcomes when compared with lower-dose diuretic therapy^[8].

Intravenous inotropes have also not improved outcomes in patients admitted with heart failure. In one of the first studies of chronic heart failure patients admitted with acute volume overload, the Outcomes of a Prospective Trial of Intravenous Milrinone for Exacerbations of Chronic Heart Failure (OPTIME-CHF) investigators examined the use of the positive inotrope milrinone^[9]. Nine hundred and fifty one patients admitted with chronic heart failure exacerbation were randomized to either milrinone or placebo. The primary endpoint of cumulative days of cardiovascular hospitalization in the first 60 d after randomization was similar between the two groups. Similarly, there was no difference in 60 d mortality, in-hospital mortality, or the composite of death or readmission. The use of milrinone was associated with more hypotension and new atrial arrhythmias. Perhaps more sobering, in this group of patients with NYHA functional class III and IV symptoms and a mean ejection fraction of 23%, the mean days of hospitalization for any cause within the first 60 d after

discharge was 13.5 in the placebo group and 13.4 in the milrinone group. Additionally, after discharge from their initial hospitalization, 35.3% of the placebo group and 35.0% of the milrinone group were either readmitted to the hospital or dead within 60 d. Even after their admission and “optimization” of medical therapy by heart failure experts, 9.5% of the patients enrolled in this trial were dead within 2 mo of discharge.

Nesiritide is a B-type natriuretic peptide that has been associated with a decrease in pulmonary capillary wedge pressure *via* its vasodilation and natriuresis^[10,11]. Despite only being demonstrated to be a vasodilator in clinical trials, many now, perhaps incorrectly, use nesiritide as a first line diuretic. Wang *et al*^[12] demonstrated that this might not be the correct use for the drug. In a small trial of 15 patients hospitalized for heart failure with mild renal insufficiency (baseline creatinine of 1.8 mg/dL), they performed a double-blind, placebo-controlled, crossover study. Patients were randomized to receive either placebo or nesiritide for 24 h on consecutive days. There were no differences in glomerular filtration rate, renal plasma flow, urine output, or sodium excretion for the patients between the two agents. Sackner-Bernstein *et al*^[13] also conducted a meta-analysis of three randomized controlled trials that suggests nesiritide may be associated with a higher risk of death compared to vasodilators and diuretics. Controversy still exists over nesiritide's deleterious effects on renal function and short-term mortality. More recent trials have demonstrated similar safety endpoints, but no clear benefit to nesiritide therapy. The Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure Trial evaluated the utility and safety of nesiritide in a randomized controlled trial of 7141 patients. Though there was no significant difference in rate of all cause mortality or worsening renal function, there was also only a small, non-significant change in patient dyspnea and no effect on rehospitalization rate^[14]. The recently published Renal Optimization Strategies Evaluation in Acute Heart Failure, which was also presented at the American Heart Association 2013 Annual Scientific Session Late Breaking Clinical Trials, also failed to show benefit of low dose nesiritide. This multicenter randomized trial showed no difference in 72 h urine volume, cystatin C levels changes, symptom relief or concomitant diuretic dose needs. Though there was no difference in renal function or death, there was increased incidence of hypotension in the nesiritide group^[15].

Clearly, the currently available agents for the treatment of heart failure in the acute setting are not associated with satisfactory outcomes. The rest of this paper will review a newer class of agents, arginine vasopressin antagonists, for the therapy of this deadly syndrome.

ARGININE VASOPRESSIN: PATHOPHYSIOLOGY

AVP is a neurohypophyseal peptide that serves the roles of vasoconstrictor and body water regulator. Turner *et al*^[16] were the first to isolate and synthesize vasopressin in 1951. Synthesized in the paraventricular and supraoptic nuclei of the hypothalamus and stored in the posterior pituitary

Table 1 Location and effect of vasopressin receptors^[13,17,21,23-25]

Receptor subtype	Location	Action	Cardiovascular end effects
V _{1A}	Liver, vascular smooth muscle, platelets, adrenal cortex, kidney, spleen, adipocytes, reproductive organs, brain, lung	Vasoconstriction	Left ventricular hypertrophy and remodeling, increase in afterload, myocyte hypertrophy
V _{1B}	Corticotroph cells, pancreas, adrenal medulla, possibly kidney	Release of adrenocorticotrophic hormone	May mediate release of aldosterone
V ₂	Renal collecting ducts	Antidiuresis <i>via</i> increased water permeability	Hyponatremia, edema, increase in preload, pulmonary vascular congestion and left sided filling pressures

gland, vasopressin is released in response to osmotic and non-osmotic forces. AVP's release is sensitive to changes in osmolality. Osmoreceptors in the hypothalamus stimulate increased AVP secretion after sensing as little as a 1% increase in serum osmolality. A decrease in 5% to 10% of plasma volume is required for AVP release, stimulated *via* baroreceptors that sense a low volume state^[17].

Three different vasopressin receptors have been isolated: V_{1a}, V_{1b} (also known as V₃) and V₂ receptors (Table 1). The V_{1b} receptor is expressed in the anterior pituitary gland and pancreatic islet cells, and although it does not have a major role in CHF, it may mediate release of aldosterone *via* modulation of adrenocorticotropin hormone release^[18]. The V_{1a} receptor (V_{1aR}) is present in blood vessels and the kidney, where stimulation is responsible for vascular constriction and possibly regulation of water reabsorption, respectively. V_{1aR} is a G_q-protein coupled receptor and, *via* phosphatidylinositol hydrolysis, stimulates mobilization of intracellular calcium. V_{1aR} knockout mice have a blunted response to AVP-induced vasoconstriction and decreased sympathetic activity^[19]. Additionally, they have lower levels of aldosterone, renin and angiotensin II as well as higher urine output. V₂ receptors are present in the thick ascending limb of the loop of Henle and collecting ducts of the renal tubular system. *Via* G_s-protein coupled receptor signaling and subsequent activation of adenylate cyclase, cyclic adenosine monophosphate levels increase and cause translocation of the water channel aquaporin-2 (AQP2), thereby increasing water permeability, reducing the rate of free water secretion and concentrating the urine^[17,20]. This causes a decrease in urine production that has been found to be proportional to the concentration of plasma vasopressin.

AVP IN HEART FAILURE AND HYPONATREMIA

AVP levels are elevated in congestive heart failure patients^[21,22]. Investigators in the SOLVD trial found that AVP was significantly elevated in asymptomatic patients with left ventricular dysfunction (ejection fraction less than 35%) when compared to controls, and even more so elevated in symptomatic patients with left ventricular dysfunction^[6]. When plasma osmolality increases in both control and CHF patients, there is a significant exaggerated AVP response in CHF patients^[23]. Although known to primarily be produced in the hypothalamus, vasopressin has also been found in

isolated rat hearts undergoing the stress of acute pressure overload or nitric oxide stimulation^[24].

AVP leads to worsening heart failure by a variety of mechanisms. Activation of V_{1aR} causes arteriolar vasoconstriction resulting in increased systemic vascular resistance and afterload. At higher physiologic AVP levels, V_{1aR} also mediates coronary vasoconstriction, thus decreasing coronary blood flow and cardiac contractility^[18,24]. Stimulation of rat cardiac fibroblasts with AVP leads to cellular hypertrophy and proliferation *via* activation of the V_{1aR}^[25,26]. AVP-stimulated rat myocytes also express increased levels of ANP, a marker of hypertrophy^[25]. The end result of AVP binding to V_{1aR} is left ventricular hypertrophy and remodeling *via* vasoconstriction, increase in afterload, and myocyte hypertrophy.

V_{2R} stimulation primarily leads to free water retention, which in turn causes an increase in preload, pulmonary vascular congestion and left sided filling pressures. The low output state of heart failure results in V_{2R} activity, with nonosmotic stimulation of vasopressin release predominating, despite hypotonicity. In experimental CHF induced in a rat model, Xu and colleagues found increased AVP levels and increased AQP2 channel expression in the apical membrane of collecting ducts when compared with controls. When the CHF rats were treated with a V_{2R} vasopressin antagonist, OPC 31260, they had increased aquaresis and plasma osmolality as well as decreased AQP2 expression^[27].

In addition to hemodynamic alteration, increased water permeability *via* AQP2 channels leads to edema and hyponatremia^[22]. Hyponatremia is a marker for advanced disease and poor outcome in CHF. In a retrospective analysis of OPTIME-CHF, patients with sodium levels in the lowest quartile had higher 60 d mortality and rehospitalization rates when compared to patients with higher sodium levels^[28]. The presence of hyponatremia also limits the use of diuretics, as these agents only exacerbate loss of sodium, and ACE inhibitors, since hyponatremia is an independent risk factor for decline in renal function during treatment with such agents^[29]. Hyponatremia is treated primarily *via* difficult to adhere to free water restriction.

AVP ANTAGONISM IN ADHF

Recently, specific antagonists to vasopressin have been developed as potentially useful agents for patients with heart failure and hyponatremia. In theory, antagonism of the

Table 2 Summary of key studies of vasopressin antagonists

Vasopressin antagonist	Study	Design	Endpoint	Results
Lixivaptan (VPA985)	Martin <i>et al</i> ^[30] , 1999	21 NYHA II and III patients randomized to placebo <i>vs</i> one of four doses (30, 75, 150, 250 mg)	Urinary AQP-2 excretion	Decrease in urinary AQP-2 excretion, increased solute-free water clearance and urine output, decreased urinary osmolality
	Wong <i>et al</i> ^[32] , 2003	44 hyponatremic patients randomized to placebo <i>vs</i> one of three doses (25, 125, or 250 mg bid) over a 7-d inpatient stay	Correction of hyponatremia	Increased free water clearance and serum sodium
	Abraham <i>et al</i> ^[31] , 2006	42 patients with mild to moderate CHF randomized to placebo <i>vs</i> ascending single-dose drug (10-400 mg)	24 h urine volume and serum sodium	Increased urine volume at 4 h and 24 h, increased serum sodium at higher doses
	BALANCE ^[33]	650 CHF patients randomized to placebo <i>vs</i> lixivaptan	Correction of hyponatremia	Increased serum sodium levels
Conivaptan	Udelson <i>et al</i> ^[41] , 2001	142 NYHA III and IV patients randomized to placebo <i>vs</i> single IV-dose (10, 20, 40 mg)	Effect on hemodynamic parameters	Reduced PCWP, RAP
	Goldsmith <i>et al</i> ^[42] , 2008	Dose-ranging pilot study of IV conivaptan in 170 randomized patients with worsening CHF	Assessment of global and respiratory status	Increased urine output
	Russell <i>et al</i> ^[43] , 2003	143 patients randomized to placebo <i>vs</i> one of three po doses	Effect on urine output	No change in status
	Zeltser <i>et al</i> ^[45] , 2007	84 euvolemic or hypervolemic hyponatremic patients randomized to placebo <i>vs</i> IV conivaptan for 4 d (40 or 80 mg/d)	Change in time to reach 70% of peak O ₂ consumption	Increased urine output
	Annane <i>et al</i> ^[46] , 2009 (The Conivaptan Study Group)	83 euvolemic or hypervolemic hyponatremic patients randomized to placebo <i>vs</i> po conivaptan for 5 d (40 or 80 mg/d)	Change in serum sodium, measured by area under the sodium-time curve	No change in exercise endpoint
Tolvaptan (OPC-41061)	Gheorghade <i>et al</i> ^[47] , 2003	254 patients randomized to placebo <i>vs</i> 30, 45 or 60 mg/d for 25 d	Change in serum sodium, measured by area under the sodium-time curve	Increased serum sodium
	Gheorghade <i>et al</i> ^[48] , 2004 (ACTIV CHF)	Phase II study in 319 patients randomized to placebo <i>vs</i> 30, 60, or 90 mg/d for 60 d	Change in body weight at 24 h	Decreased body weight, increased urine output, increased serum sodium, decreased edema
	Gheorghade <i>et al</i> ^[42,50] , 2007 (EVEREST)	Large 4133 patient multi-center randomized study of short and long term effects of tolvaptan in ADHF	Heart failure outcomes	Significant decrease in body weight at 24 h
	Udelson <i>et al</i> ^[54] , 2007 (METEOR)	240 patients, NYHA II or III, randomized to placebo <i>vs</i> tolvaptan	CHF symptoms	No change in worsening heart failure at 60 d
	Udelson <i>et al</i> ^[53] , 2008	181 patients, NYHA III and IV, randomized	Mortality and heart failure related morbidity	Improvement in some CHF symptoms
			LVEDV	No difference in long-term mortality or morbidity
			Hemodynamic effects	No change in LVEDV at one year
				Decreased PCWP, RAP, PAP

IV: Intravenous; PO: Oral; PCWP: Pulmonary capillary wedge pressure; RAP: Right atrial pressure; O₂: Oxygen; CHF: Congestive heart failure; ADHF: Acute decompensated heart failure; LVEDV: Left ventricular end diastolic volume; PAP: Pulmonary artery pressure.

V1aR, V2R or both may be beneficial in patients with heart failure. There are many different vasopressin antagonists, and some have been evaluated in patients with heart failure as outlined in Table 2.

Lixivaptan

The first agent studied was lixivaptan (or VPA-985, Cardiokine Inc, Philadelphia, PA), an oral, V2R selective, vasopressin antagonist. Martin and colleagues reported administering this agent at four different doses to 21 chronic NYHA functional class II and III patients and found decreased urinary AQP2 secretion (a marker of AVP action), increased solute-free water clearance and urine output, and decreased urine osmolality^[30]. These results were confirmed in a single-ascending-dose 42 patient study of safety, efficacy, and tolerability of lixivaptan by the same authors^[31]. Wong *et al*^[32] also found a dose-dependent increase in sodium concentrations amongst 44

hyponatremic patients (six of which had CHF) receiving VPA-985. Higher doses (250 mg) caused dehydration and increases in vasopressin levels.

Abraham and colleagues further studied the role of lixivaptan in three phase III clinical trials. The LIBRA and HARMONY trials demonstrated safety of initiation and efficacy of lixivaptan in patients with euvolemic hyponatremia in the inpatient and outpatient settings, respectively^[33,34]. The BALANCE (Treatment of Hyponatremia BAsed on LixivAptan in NYHA Class III/IV Cardiac Patient Evaluation) Trial was specific to hospitalized heart failure patients. The BALANCE trial was a large, international, multicenter, randomized, placebo-controlled, double blind study of 650 hospitalized CHF patients with serum sodium < 135 mEq/L. The primary endpoint was correction of hyponatremia, with additional endpoints including dyspnea, cognitive function

and days of hospital-free survival^[35]. Patients were treated with 50-100 mg of lixivaptan a day, twice daily, for 60 d. Though results have not been published, they were presented at an Federal Drug Administration (FDA) advisory committee meeting in 2013^[36]. At seven days, there was a significant increase in serum sodium in the lixivaptan versus placebo group (2.5 mEq/L *vs* 1.3 mEq/L, $P = 0.001$). There was a nonsignificant early increase in mortality in the lixivaptan group however overall death rates and hospitalization rates were no different from the placebo group. In an extension study, long-term safety of lixivaptan was studied in a 28-wk open label study, results of which have not been published^[37,38]. Lixivaptan is not yet FDA approved.

Conivaptan

Binding of V1aR by vasopressin plays an important role in cardiac contractility and remodeling. Therefore, a dual receptor (V1a and V2) antagonist, conivaptan (YM087), has also been evaluated in heart failure. Early experimental studies in animals showed the utility of intravenous conivaptan. In a canine model of AVP-induced CHF, infusion of intravenous conivaptan corrected poor cardiac hemodynamics^[39]. In rats with post myocardial infarction CHF, intravenous conivaptan not only significantly improved right ventricular systolic pressure, left ventricular end-diastolic pressure, lung/body weight and right atrial pressure, but also, when compared to a V2 selective antagonist, increased the first derivative of left ventricular pressure, a measure of cardiac contractility^[40].

One hundred, forty-two patients were randomized to either placebo or an intravenous dose of conivaptan at one of 3 different doses^[41]. These patients had NYHA III or IV functional symptoms, but were stable outpatients that were admitted for placement of a right heart catheter and infusion of study drug. The investigators found a significant reduction in pulmonary capillary wedge and right atrial pressure. Additionally, urine output increased by 176 ± 18 mL/h in the high dose conivaptan group, without affecting systemic blood pressure, heart rate or serum electrolytes, including serum sodium. The beneficial hemodynamic effects of conivaptan therefore may be generalizable to CHF patients, and not just those with hyponatremia. Goldsmith and colleagues studied the use of intravenous conivaptan in a pilot study of 170 hospitalized patients with acute decompensated heart failure^[42]. Their randomized placebo-controlled multi-center trial administered conivaptan for 48 h (as opposed to 12 h in the former study) alongside loop diuretics and found an average 1.0 to 1.5 L/d increase in urine output. They did not measure hemodynamics, however they found no significant change in systemic blood pressure.

The oral formulation of conivaptan has also been investigated^[43]. In a 12-wk study in patients with NYHA class II-IV symptoms, 343 patients were randomized to either placebo or one of three doses of oral conivaptan. Using the hypothesis that a dual vasopressin receptor blocker would cause pulmonary vasodilatation and aquaresis and a subsequent improvement in submaximal exercise, the

primary endpoint of the study was a change in the time to reach 70% of peak oxygen consumption^[44]. However, there were no differences in any exercise endpoints between the placebo arm and the three groups of patients on different doses of conivaptan.

Conivaptan has also proven efficacy in treatment of hyponatremia. Intravenous conivaptan administered to euvoletic and hypervolemic hyponatremic patients significantly increased serum sodium concentrations 9.4 ± 0.8 mEq/L at a conivaptan dose of 80 mg/d after four days of treatment^[45]. Oral conivaptan showed similar results in a study of 84 hyponatremic patients (33% had CHF), with an increase in sodium of 9.1 ± 0.9 mEq/L at the end of five days of treatment with 80 mg/d of oral conivaptan^[46].

Intravenous conivaptan (Vaprisol, Astellas Pharma US, Inc.) was the first Federal Drug Administration (FDA) approved vaptan for the treatment of euvoletic hyponatremia. It is currently approved for treatment of euvoletic and hypervolemic hyponatremia in hospitalized patients, including those with heart failure. Administration involves an additional loading dose of 20 mg IV over 30 min followed by a 20 mg continuous infusion over 24 h. Metabolism is *via* cytochrome P450 3A4. Conivaptan is not directly approved for the treatment of acute heart failure without hyponatremia. There are no planned future trials studying oral conivaptan.

Tolvaptan

Tolvaptan (OPC-41061), a V2 selective vasopressin receptor antagonist, has been the most studied drug of its class in patients with heart failure. Gheorghade *et al*^[47] reported the results of a study of 254 patients who were randomized to either placebo or three different doses of tolvaptan for 25 d. The primary endpoint was change in body weight. Additional endpoints included urine sodium excretion, urine volume, urine osmolality, and ankle edema measurements. A decrease in body weight of about 1 kg was found after the first day that was maintained throughout the study. There was also an increase in urine volume and a normalization of serum sodium with tolvaptan.

A second dose ranging phase II study, ACTIV in CHF (Acute and Chronic Therapeutic Effect of a Vasopressin Antagonist in Congestive Heart Failure), was performed with a primary endpoint of change in body weight at 24 h^[48]. Additionally, heart failure outcomes including death, hospitalization, or unscheduled visits for heart failure at 60 d were collected. Body weight at 24 h after tolvaptan administration decreased by 1.8 kg, 2.1 kg, and 2.05 kg in the 30, 60, and 90 mg per day arms compared to a decrease of 0.6 kg in the placebo arm. This decrease occurred without a change in renal function or hypokalemia. There was no difference in the secondary outcome of worsening heart failure at 60 d. Additionally, there was an increase in serum sodium in the tolvaptan arms. Although the study was not powered for mortality, a post-hoc analysis of patients with high blood urea nitrogen levels or severe congestive symptoms demonstrated a statistically higher mortality rate in placebo *vs* tolvaptan treatment groups.

Study of Ascending Levels of Tolvaptan in Hyponatremia

1 and 2 (SALT-1 and SALT-2) were two simultaneous phase III trials published in 2006 by Schrier *et al*^[49]. Patients with euvolemic and hypervolemic hyponatremia demonstrated an increase in serum sodium levels by day 4 and sustained at day 30. Hyponatremia recurred when the drug was discontinued after the 30-d treatment period.

The Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study With Tolvaptan (EVEREST) Trial was a large event-driven, randomized, double-blind, placebo-controlled study of 4133 patients hospitalized with acute heart failure. Patients were randomized to placebo or a minimum of 60 d of tolvaptan at 30 mg/d. Short-term clinical effects were examined in two identical trials of 2048 and 2085 patients^[50]. There were statistically significant differences in endpoints for body weight and dyspnea in both trials' tolvaptan arms when compared to placebo, significant decrease in edema in only one trial, and no change in global clinical status in either trial. There was also greater improvement in other physician-assessed CHF signs and symptoms such as dyspnea, edema, orthopnea and jugular venous distention ($P < 0.05$) as well as increased serum sodium levels in the tolvaptan group^[51]. Patients receiving tolvaptan were discharged on lower doses of furosemide. Although the short-term trials from the EVEREST group showed improvement in congestion without significant adverse effects, the long-term outcome trial did not show any benefit on all-cause mortality or heart failure-related morbidity^[52]. In a post-hoc analysis of the EVEREST trial studying the effect of QRS duration on heart failure outcomes, a prolonged QRS interval was associated with poorer outcomes however use of tolvaptan did not affect these endpoints^[53].

Effects of tolvaptan on hemodynamics and left ventricular physiology have also been assessed. The METEOR (Multicenter Evaluation of Tolvaptan Effects on Left Ventricular Remodeling) Trial, which investigated tolvaptan versus placebo in 240 patients with NYHA class II or III symptoms and left ventricular ejection fraction less than or equal to 30%, showed no difference in the primary end point of left ventricular end diastolic volume at the end of one year^[54]. Although not powered for such outcomes, the study did show a decrease in morbidity and mortality in the tolvaptan-treated patients. In order to further substantiate the findings that tolvaptan improved congestion, Udelson and colleagues assessed the acute hemodynamic effects of tolvaptan in 181 patients with symptomatic heart failure (NYHA class III or IV)^[55]. They found that tolvaptan effectively decreased pulmonary capillary wedge pressure, right atrial pressure and pulmonary arterial pressure.

Similar to other vasopressin antagonists, tolvaptan improves serum sodium concentrations in hyponatremic patients^[49]. In a study of 448 patients with euvolemic or hypervolemic hyponatremia, tolvaptan significantly increased sodium levels at day 4 and day 30. Interestingly, though EVEREST was a study specifically of patients with acutely decompensated heart failure, only about 8% of patients had significant hyponatremia (< 134 mEq/L)^[52]. In this subset of patients however, there was a significant rise in serum sodium seen as early as day 1.

Tolvaptan (Otsuka, Inc.) is the only oral vasopressin antagonist that is FDA approved^[56]. Tolvaptan is approved specifically for treatment of euvolemic or hypervolemic hyponatremia (per FDA label, "serum sodium < 125 mEq/L or less marked hyponatremia that is symptomatic and has resisted correction with fluid restriction"). Starting dosage is typically 15 mg/d and can be increased to 30 mg/d at the second dose but should not exceed 60 mg/d. Initially approved for longer term treatment, due to liver failure observed in a study of patients with underlying cirrhosis, the FDA revised its label in April 2013 and limits treatment duration to 30 d^[57]. It should be noted that in the SALTWATER Trial, an open-label extension of the SALT-1 and SALT-2 Trials, in which patients with hyponatremia were treated with tolvaptan for a mean duration of 701 d, six patients experienced drug-related adverse effects, all related to sodium levels^[58]. Tolvaptan should be initiated or re-initiated only in hospitalized patients where serum sodium levels can be closely monitored. Serum sodium should not be too rapidly corrected (faster than 12 mEq/24 h) as this can lead to neurologic effects such as dysarthria, mutism, dysphagia, lethargy, affective changes, spastic quadriparesis, seizures, coma and death. Tolvaptan is primarily metabolized by cytochrome P450 3A4, and therefore attention should be paid to potential drug interactions. As with other vaptans, side effects include thirst, dry mouth and frequent urination.

CLINICAL IMPLICATIONS

Vasopressin antagonists are a new group of drugs that provide effective aquaresis without effecting morbidity or mortality. Thus far, the vaptans are approved only for use in treatment of hyponatremia (with or without hypervolemia). Additionally, most studies with CHF patients have included patients only with reduced, and not preserved, systolic function. Although not yet fully studied, these drugs may prove to be beneficial for the treatment of heart failure as a replacement for or in conjunction with diuretics. They do not cause hypokalemia and do not appear to be associated with upregulation of the neurohormonal system. Although these agents have not been shown to reduce mortality in the long term, perhaps their use will allow one to administer lower diuretic doses resulting in less electrolyte disturbance and improved patient safety. Currently, use of a vaptan may be considered in volume overloaded patients who either have or are developing hyponatremia. Routine use of vasopressin antagonists has currently not been shown to be beneficial.

When considering the initiation of a vaptan, it is important to think about duration of therapy, route of administration and whether these medications should be used in the inpatient or outpatient setting. Most studies have only used these medications for short durations and there is limited data to support long-term use of vaptans. Therefore, although the effective increase in serum sodium concentration has been shown to last through treatment duration, it must be emphasized that these medications have only short term effect in their current role, and do not by any means aim to cure the underlying disease process.

Although vaptan use has been theorized to be beneficial in the short-term acute setting, many of the studies were done using stable outpatients. It is unclear how useful these drugs will be in acutely decompensated heart failure. The most thoroughly studied of these medications, tolvaptan, is an oral agent, and therefore absorption may be affected by gut edema. One possibility is for patients to acutely receive intravenous conivaptan as inpatients and then be transitioned over to oral tolvaptan, on which they may go home for a short duration such as 30 d. Lixivaptan is an additional promising oral agent that is not yet FDA approved. Vasopressin antagonists should only be initiated inpatient so that sodium levels can be closely monitored and rapid correction, which can be detrimental, can be avoided.

CONCLUSION

Further investigation of vasopressin antagonists is needed in patients with both preserved and reduced ejection fractions. Acute heart failure has been a challenge to treat thus far. Results from ongoing studies of vasopressin antagonists may change the treatment approach in both inpatient and outpatient heart failure patients. However, there is still work to be done to decrease long-term morbidity and mortality in this patient population, which the vaptans do not seem to promise.

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Implications of Klotho in vascular health and disease

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Abstract

Cardiovascular disease (CVD) is a prevalent condition in general population and the first cause of death overall. Klotho, a pleiotropic protein related to longevity that acts as a co-receptor of the fibroblast growth factor 23, has been proposed as a key regulator of the development of CVD. In the few clinical studies made, it has been observed a relationship between low levels of soluble Klotho and the occurrence and severity of CVD, as well as a reduction of cardiovascular risk when they are high. Also, different polymorphisms of human Klotho gene have been related to the incidence of cardiovascular events. Moreover, several experimental studies indicate that this protein acts in the maintenance of vascular homeostasis. Klotho improves endothelial dysfunction through promotion of NO production and mediates anti-inflammatory and anti-aging effects such as suppression of adhesion molecules expression, attenuation of nuclear factor-kappa B or inhibition of Wnt signaling. Furthermore,

this protein is related to the attenuation of vascular calcification as well as prevention of cardiac hypertrophy. The expression of this protein in the vascular wall implies a new scenario for the treatment of vascular disorders. The purpose of this review is to provide an overview of the relationship between the Klotho protein and CVD, in addition to its role in the maintenance of functional vascular integrity.

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Key words: Klotho; Cardiovascular disease; Vascular health; Aging; Endothelial dysfunction; Vascular calcification

Core tip: Cardiovascular disease (CVD) is the first cause of death worldwide. The anti-aging factor Klotho has been linked to the development of CVD since clinical studies relate circulating levels of Klotho with the appearance of vascular disease and different *Klotho* gene variants are associated with increased cardiovascular risk. Furthermore, Klotho is involved in promotion of vascular health through different mechanisms. The recent description of its expression in vascular tissue opens up new options for the treatment of cardiovascular diseases.

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INTRODUCTION

The cardiovascular disease (CVD) is highly prevalent in the general population and the leading cause of death worldwide^[1], maintaining these projections in the future^[2]. CVD broadly comprises coronary artery disease (CAD),

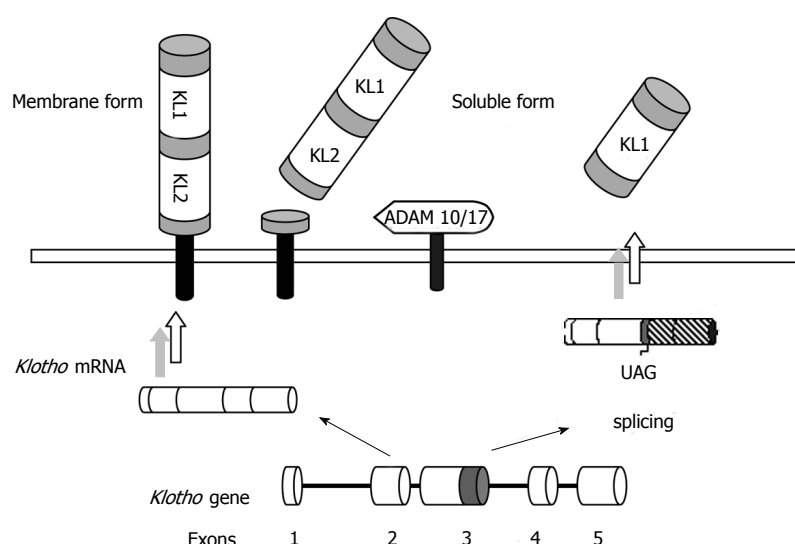


Figure 1 Mechanisms of generation of the different forms of Klotho. ADAM: Membrane-anchored A Desintegrin and metalloproteinase.

myocardial infarction, vascular stiffening and left ventricular hypertrophy^[3].

Klotho, a gene originally identified in 1997 codifying for a novel anti-aging protein, has been implicated in a multitude of biological processes, most of them related to human longevity^[4]. Mice lacking the *Klotho* gene develop a phenotype similar to premature human aging, which includes endothelial dysfunction, vascular calcification, progressive atherosclerosis and shortened lifespan^[5]. A reduction in Klotho levels is observed in chronic kidney disease (CKD) patients, similar to other premature vascular aging diseases, such as hypertension or diabetes mellitus. Even normal aging is associated with a reduction in serum and urine concentration of Klotho^[6-8].

The first function described for Klotho is its role in the metabolism of phosphorus as the obligatory co-receptor of fibroblast growth factor 23 (FGF23), a bone-derived hormone responsible of the phosphate balance in the body through promotion of renal phosphate excretion. Klotho directly binds to FGF receptors (FGFRs) constituting a high affinity complex for FGF23 which mediates the intracellular effects of this phosphatonin^[9]. More recently, the involvement of Klotho in vascular protection through different mechanisms has been demonstrated. These mechanisms include inhibition of oxidative stress, modulation of inflammation or attenuation of vascular calcification^[10-12]. Therefore, Klotho has been suggested as a master regulator of CVD^[13]. The aim of this review is to provide an overview of what is known so far about Klotho and its relationship with CVD, besides its role in the maintenance of vascular homeostasis.

MOLECULAR CHARACTERISTICS OF KLOTHO

The human *Klotho* gene comprises 5 exons and is located in a region of approximately 50 kb on chromosome 13q12. This gene encodes for two possible transcripts: a full-length, translated into a single-pass transmembrane protein of 1012 amino acids (130 kDa), or an alternative spliced transcript, which encodes the N-terminal half

of 549 amino acids (65-70 kDa) and is secreted to the extracellular space. Another form of soluble Klotho can also be generated through proteolytic cleavage of the transmembrane form by membrane-anchored A Desintegrin and metalloproteinase (ADAM) -17 and ADAM-10, so that the full-length extracellular domain is released into the circulation^[14-17] (Figure 1). Soluble Klotho predominates in humans over the membrane form and is detectable in urine, serum and cerebrospinal fluid^[18]. This circulating form acts as a humoral factor with multitude of functions such as anti-oxidation, modulation of renal ion channels, anti-Wnt signaling or anti-apoptosis and senescence effects^[19].

The Klotho protein comprises an extracellular domain composed of two repeat sequences (KL1 and KL2), two short membrane-spanning regions (21 amino acids) and an intracellular carboxyl (11 amino acids) domain. The KL1 and KL2 sequences share 20%-40% sequence identity with the Family 1 glycosidases^[4,16].

In humans, *Klotho* is mainly expressed in the kidneys, but its tissue distribution also includes brain, reproductive organs, pituitary gland, parathyroid glands, urinary bladder, skeletal muscle, placenta, thyroid gland, colon^[4], and more recently described, human vascular tissue^[20,21]. The membrane form mainly acts as the obligatory co-receptor for FGF23, thereby tissues expressing Klotho are potential targets for FGF23 to exert its actions^[9,22,23].

CLINICAL ASSOCIATIONS OF KLOTHO AND CVD

Serum Klotho and CVD

Although the circulating levels of soluble Klotho have been initially proposed as biomarker of renal function, since some works show a decrease in serum levels during development of CKD^[6], its association with cardiovascular risk has been less extensively explored.

In a first work, Semba *et al.*^[24] found that in community-dwelling adults higher plasma Klotho concentrations are independently associated with a lower likelihood of having CVD, defined as CAD, heart failure stroke, or

peripheral arterial disease. Likewise, in a recent study developed by our group, we observed that patients with significant CAD have lower soluble concentrations of soluble Klotho, as well as a reduced expression level of *Klotho* mRNA in the vascular wall. Besides, the reduced serum Klotho levels and decreased vascular gene expression were associated with the presence and severity of CAD independently of established cardiovascular risk factors such as age, diabetes, hypertension, smoking, dyslipidemia, and inflammation^[25].

Moreover, Kitagawa *et al.*^[26] observed that serum Klotho level is an independent determinant of marked arterial stiffness but not of other types of vascular dysfunction such as atherosclerosis, endothelial dysfunction or vascular calcification, in CKD patients^[26]. In contrast, in a very recent work, Seiler *et al.*^[27] found no significant relationship between soluble Klotho and cardiovascular outcomes in a CKD stages 2-4 cohort.

Taken together, these studies suggest that a reduction in the levels of soluble Klotho may promote or encourage the development and progression of CVD, while high levels of this factor prevents the risk of CVD. In any case, further studies are needed to clarify the relationship between circulating Klotho levels and cardiovascular risk.

Genetic variation of Klotho and CVD

Genetic variation studies have demonstrated that *Klotho* gene polymorphisms might be associated with longevity^[28] and CAD^[29-32]. In particular, the KL-VS allele, characterized by six SNPs in a region of 800 bp in exon 2 and flanking sequence, is prevalent in the population and is associated with a reduced longevity^[28]. In a study where two different groups of healthy siblings were tested, Arking *et al.*^[29] found that this functional variant of *Klotho* gene is an independent risk factor for CAD. The risk associated with this allele is modulated by modifiable risk factors, such as hypertension, increased high-density lipoprotein cholesterol levels or smoking^[29]. Likewise, in an Ashkenazi Jew group it was found that homozygous KL-VS individuals were at higher risk of stroke than wild-type subjects^[33].

In the case of G-395A polymorphism, the A allele has been found to be an independent predictor of atherosclerotic CAD but not of vasospastic angina in Japanese population^[30]. This polymorphism affects the promoter of the *Klotho* gene, so that the G→A substitution impairs protein binding to the region and consequently affects gene expression^[34] and soluble Klotho levels. Similarly, Jo *et al.*^[32] observed an association of the G-395A allele with CAD but not with coronary artery calcification in Korean patients. Besides, subjects with the T allele for the C1818T polymorphism (located in exon 4) have lower prevalence of CAD than those with CC genotype^[31].

MECHANISMS OF VASCULAR PROTECTION

Endothelial dysfunction (NO production)

One of the first vasculoprotective activities described

for Klotho is its role in maintenance of endothelial homeostasis. *Kl^{-/-}* mice show attenuated aortic and arteriolar vasodilatation, which can be increased after two weeks of parabiosis with wild type mice^[35]. Moreover, these *Kl* heterozygous mice show a significant reduction of urinary excretion of NO₂⁻ and NO₃⁻ (NO metabolites), suggesting a decrease in NO production^[35]. In Otsuka Long-Evans Tokushima Fatty rats, an animal model which displays multiple atherogenic risk factors, adenovirus-mediated *klotho* gene delivery results in improvement of aortic relaxation and increased NO production^[36]. These findings point to a direct involvement of Klotho in improving endothelial dysfunction through pathways involving NO. Consistent with this, Shimada *et al.*^[37] observed impaired angiogenesis, a NO-dependent process, and reduced endothelium-derived NO release in *kl/kl* mice.

This reduction of NO mediated by Klotho deficiency can be due to its accelerated degradation because of increased oxidative stress associated with aging. Klotho is able to increase resistance to oxidative stress inducing expression of manganese superoxide dismutase (Mn-SOD) through activation of FoxO forkhead transcription factor^[38]. In regard of this, Klotho increases Mn-SOD activity and NO production *via* c-AMP-PKA-dependent pathway in human umbilical vascular endothelial cells (HUVECs)^[10], and it also reduces H₂O₂-induced apoptosis and cellular senescence^[39]. Likewise, Klotho transfection of cultured vascular smooth muscle cells (VSMCs) also reduces superoxide production and decrease angiotensin II-induced oxidative stress^[40].

Another possibility is that Klotho regulates expression levels of the endothelial NO synthase (eNOS). Six *et al.*^[41] recently observed that attenuation mediated by Klotho of FGF23 or phosphate-induced vasoconstriction is abolished by adding nitro-L-arginine, a competitive inhibitor of NOS. Moreover, they observed that exposure of HUVECs to Klotho increased NO production and induced eNOS phosphorylation and iNOS expression. Interestingly, Klotho was able to increase H₂O₂ production in cultured human VSMCs (HVSMCs), which suggests a more complex effect of this protein on the regulation of vascular tone through mediation of a ROS/NO balance^[41].

Aging and inflammation

Inflammation is a central process in CVD^[42,43] and Klotho has been suggested to play a protective role in the vessels since it mediates anti-inflammatory actions. In cultured HUVECs, incubation with Klotho results in suppression of expression of cell adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)^[11]. These Klotho effects in ECs also include attenuation of the activation of NF- κ B and blockade of tumor necrosis factor- α induced monocyte adhesion^[11]. Likewise, the intracellular form of Klotho is capable to inhibit RIG-I-induced expression of interleukin (IL)-6 and IL-8 both *in vitro* and *in vivo*^[44].

Moreover, it is known that soluble form of Klotho is able to bind to various members of Wnt family, and

thereby suppress Wnt biological activity^[45,46]. Although this signal is essential for stem cells proliferation, continued activation of Wnt can contribute to cellular depletion and accelerated cellular senescence^[47]. Therefore, Klotho could exert an anti-aging effect by attenuation of Wnt signaling, preventing cellular senescence^[48].

Vascular calcification

Vascular calcification (VC) is one of the major complications of CKD and is associated with mineral and bone disorders. Since CKD patients, who have low levels of Klotho protein, and Klotho-deficient animals develop medial vascular calcification^[4], the absence of this protein has been associated with the appearance of VC. Initially, the involvement of Klotho in the protection against VC was believed to be related to its role in the regulation of phosphate metabolism as co-receptor for FGF23. However, in recent years Klotho has shown to have direct effects on vasculature to prevent this pathology.

High levels of extracellular Pi induce mineralization of VSMCs through inorganic Pi influx mediated by cotransporters NaPi type 3 (Pit-1 and Pit-2)^[49]. This process is accompanied by overexpression of osteogenic markers, such as RunX2, which leads to dedifferentiation of VSMCs^[50,51]. In 2011, Hu *et al.*^[12] found that Klotho deficiency in mouse involved increased arterial calcification, and aortic downregulation of *SM22* (a smooth muscle cell marker) expression and upregulation of the transcripts for *Pit-1*, *Pit-2* and *RunX2*. A similar expression profile was observed in the mouse model of CKD, which was prevented by Klotho overexpression. Moreover, addition of recombinant soluble Klotho to rat VSMCs cultured in high-Pi decreased aortic calcium content and Na⁺-dependent Pi uptake, confirming Klotho direct modulation of NaPi-3 activity^[12]. Administration of exogenous Klotho protein to *kl/kl* mice also attenuates aortic calcification^[52]. Therefore, it seems that Klotho prevents vascular calcification through mediation of NaPi-3 cotransporters activity and modulating VSMCs differentiation.

Consistent with this, Lim *et al.*^[21] confirmed the importance of Klotho in arterial calcification in a study where they found that silencing of Klotho in human aortic smooth muscle cells (HA-SMCs) leads to increased calcification^[21]. Interestingly, treatment with vitamin D receptor activators (VDRAs), such as calcitriol or paricalcitol, restores Klotho expression in pro-calcific cultured HA-SMCs and increases serum and urine Klotho in uremic mice^[21,53]. This VDRA therapy is associated with improved aortic medial calcification and increased osteopontin expression, an anticalcification factor^[53].

Cardioprotection

Cardiac hypertrophy is a high prevalent pathological condition among end stage renal disease patients, which leads to cardiac dysfunction and death^[54-56]. Stress signals induce abnormal growth and remodeling that progress to heart failure. Klotho is involved in cardioprotection since its deficiency produce an exaggerated cardiac hypertrophy

caused by isoproterenol (ISO) injection in mice^[57]. Likewise, its administration ameliorates ISO-induced structural changes in mouse hearts, *e.g.*, disordered arrangement of myocardial fibers, fibroblastic hyperplasia, mononuclear cell infiltration or interstitial and perivascular fibrosis^[58].

This cardiac protection by Klotho occurs through downregulation of TRPC6 channels, whose overexpression causes aberrant cardiac development and premature death^[57]. Moreover, cardiomyocyte apoptosis is an important process in cardiac remodeling^[59] and Klotho is able to suppress it by downregulation of endoplasmic reticulum stress and ROS production^[58].

KLOTHO EXPRESSION IN THE VASCULAR WALL

In recent years, the detection of Klotho in human vascular tissue^[20,21,60] has extended the range of putative target tissues of FGF23 actions. Coexpression of two cognate FGF23 receptors, FGFR-1 and -3 in the vascular wall, along with Klotho^[21], supports this idea. Furthermore, expression of Klotho protein appears to be limited to medial layer of the vessel, since it is detected by immunohistochemistry in tunica media of healthy subjects arteries^[21] or in rat aorta^[61], and by western blotting in human VSMCs^[21]. Likewise, *Klotho* mRNA is detected in cultured HVSMCs rather than human vascular endothelial cells^[60].

However, there are conflicting data which have led to a debate about the presence of Klotho in the vascular tissue. Scialla *et al.*^[62] detected no expression of Klotho in human or mouse VSMCs, neither in mouse aortas. Moreover, Lindberg *et al.*^[63] detected only low levels of Klotho transcript in different vascular tissues (aorta, mesenteric, femoral and lung arteries) and without significant differences between wild type and *Sm22-KL^{-/-}* mice (a new experimental model with targeted deletion of Klotho in VSMCs). In this study, protein expression was undetectable in vascular tissue by immunohistochemistry or western blotting, and the absence of expression of *Egr-1* in aortas of mice after injection of FGF23 indicates the lack of a functional Klotho-FGF23 signaling complex in vascular tissue^[63]. Conversely, Fang *et al.*^[64] demonstrated vascular expression of Klotho in low-density lipoprotein-deficient (*ldlr^{-/-}*) mice. In another study, Jimbo *et al.*^[61] demonstrated expression of Klotho protein in rat aortas but not in isolated VSMCs. Furthermore, they showed that extracellular signal-related kinase 1/2, an enzyme activated by FGF23 in Klotho-expressing cells^[65], was phosphorylated by FGF23 in a dose-dependent manner in Klotho-overexpressing VSMCs but not in isolated VSMCs, suggesting that presence of Klotho only occurs in contractile VSMCs^[61].

Some studies show a decreased Klotho vascular expression in CKD, similar to early reduction of this protein in the kidney during the disease^[12]. Lim *et al.*^[21] observed a marked reduction of Klotho protein expression in arteries from patients with CKD. Furthermore, they showed that exposure of HA-SMC to uremic serum

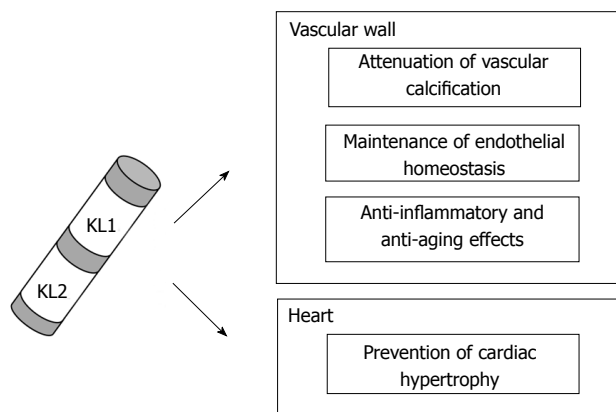


Figure 2 Mechanisms of vascular protection mediated by Klotho.

from patients with CKD, or to different conditions recalling CKD like hyperphosphatemia, hypercalcemia or proinflammatory stress, significantly reduced Klotho protein^[21]. Moreover, Fang *et al*^[64] also observed a reduction of Klotho activity in the aorta of a mice model of early CKD, although serum Klotho levels were increased. This decrease of vascular Klotho during disease could involve a FGF23 resistance state in the vascular bed. In contrast, Jimbo *et al*^[61] showed that Klotho remained unchanged in aortas of nephrectomized rats.

As already suggested, all these discrepancies can be due to differences in experimental settings, like issues regarding specificity and sensitivity of anti-Klotho antibodies, different vasculature segments analyzed or differences in cell culture conditions, as well as, variance in CKD stage^[66]. Although further studies are needed to characterize the vascular expression of Klotho in animal models, healthy subjects and CKD patients, as well as its stability under *in vitro* and *ex vivo* conditions, the set of results obtained so far seem to suggest that this tissue is sensitive to FGF23 and that CKD is a state of vascular Klotho deficiency. It is also interesting to note the relationship between the expression in human thoracic aorta tissue of vascular Klotho and ADAM-17^[20], one of the metalloproteinases responsible for the shedding of Klotho from the cell surface, which suggests the possibility that vascular wall is a source of soluble Klotho, and therefore an important element in vascular protection.

CONCLUSION

Klotho is a novel factor involved in longevity and aging, which also has a central role in regulating phosphorus metabolism acting as co-receptor for FGF23^[4,9]. But beyond these roles, several clinical studies have linked this protein to the development and progression of CVD. The reduction of circulating levels of Klotho is associated with the presence and severity of CAD and is also an independent marker of some forms of vascular dysfunction such as arterial stiffness^[25,26]. Likewise, various genetic studies have shown the association between gene variants of human *Klotho* gene with CAD or stroke^[29-32].

Klotho is involved in the protection of vasculature through various mechanisms, including prevention of endothelial dysfunction, anti-inflammatory effects, reduction of vascular calcification or attenuation of cardiac hypertrophy^[11,12,35,58] (Figure 2). The disruption in the homeostasis of this factor seems to be a key element in the development of CVD. Furthermore, Klotho expression in the vessel wall, along with the enzymes responsible for generating its soluble form^[20,21], makes the vascular context a new scenario to be considered for the treatment of vascular diseases.

The central role of Klotho in the development of CVD makes its possible use promising as a diagnostic biomarker or as a therapeutic factor for treatment of vascular diseases. However, further studies are needed to clarify the relationship between this factor and promotion of vascular health.

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Cardiac resynchronization therapy: Dire need for targeted left ventricular lead placement and optimal device programming

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Abstract

Cardiac resynchronization therapy (CRT) effected *via* biventricular pacing has been established as prime therapy for heart failure patients of New York Heart Association functional class II, III and ambulatory IV, reduced left ventricular (LV) function, and a widened QRS complex. CRT has been shown to improve symptoms, LV function, hospitalization rates, and survival. In order to maximize the benefit from CRT and reduce the number of non-responders, consideration should be given to target the optimal site for LV lead implantation away from myocardial scar and close to the latest LV site activation; and also to appropriately program the device paying particular attention to optimal atrioventricular and interventricular intervals. We herein review current data related to both optimal LV lead placement and device programming and their effects on CRT clinical outcomes.

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Key words: Heart failure; Cardiac dyssynchrony; Left

bundle branch block; Cardiac resynchronization therapy; Biventricular pacing

Core tip: Cardiac resynchronization therapy has been established as a cornerstone therapy in symptomatic patients with heart failure, severe systolic left ventricular (LV) function and widened QRS complex. In order to achieve high percentage of biventricular pacing and to reduce the number of non-responders, consideration should be given to target the optimal site for LV lead implantation away from myocardial scar and close to the latest LV site activation; and also to appropriately program the device paying particular attention to optimal atrioventricular and interventricular intervals.

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INTRODUCTION

Cardiac resynchronization therapy (CRT) is a well-established treatment strategy for patients with congestive heart failure (HF), as it has been associated with fewer hospitalizations and an improvement in left ventricular (LV) reverse remodeling, but most importantly with a prolonged survival. The recently updated guidelines recommend CRT in chronic HF patients with LV ejection fraction (LVEF) $\leq 35\%$ who remain symptomatic in New York Heart Association (NYHA) functional class II, III and ambulatory IV despite adequate medical treatment and who have left bundle branch block (LBBB) with QRS duration > 120 ms on electrocardiogram (ECG) or non-LBBB with QRS duration > 150 ms^[1]. Irrespectively of the proper patient selection according to guidelines,

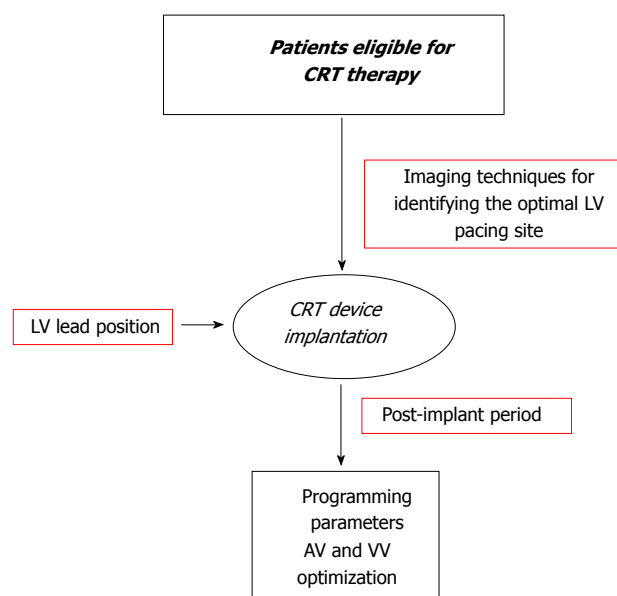


Figure 1 The factors that interact for the cardiac resynchronization response and improvement of heart failure symptoms. AV: Atrioventricular; CRT: Cardiac resynchronization; LV: Left ventric-le-ular; VV: Interventricular.

about one-third of them currently do not respond to CRT, and more than 40% do not show LV reverse remodeling^[2]. The main reasons seem to be the presence of myocardial scar, the suboptimal LV lead position, and the inadequate CRT device programming (Figure 1). The standard approach to CRT implantation consists of simultaneous (or sequential) pacing of the right ventricle (RV) and the LV *via* an epicardial coronary sinus (CS) venous branch, commonly of lateral or posterolateral location^[1]. Moreover, post-implant device programming is a first line approach to achieve the maximal benefit of biventricular pacing depending on the patients' clinical characteristics. The purpose of this article is to review and evaluate the current data related to both optimal LV lead placement and device programming and their effects on CRT clinical outcomes.

LV LEAD PLACEMENT AND OUTCOMES

Optimization of CRT aims primarily to achieve biventricular pacing as much as possible, ideally 100%, and to reduce the rate of non-responders. This is commonly related to the implantation of the LV lead, its location with respect to the anatomical location of the LV, the presence of transmural scar tissue in the pacing site, its relationship with respect to the mechanical delay, and also the number of different LV pacing sites (Figure 2). Beyond LV lead position, optimal device programming is required to eliminate the atrioventricular (AV) and the interventricular (VV) dyssynchrony by configuring the respective delays. It is well known that patients with true LBBB pattern in the baseline ECG have more favorable clinical outcomes after CRT compared to those with non-LBBB morphology. Electrical conduction delays in the LV and RV produce LBBB and RBBB pattern, respectively^[3]. Factors that may

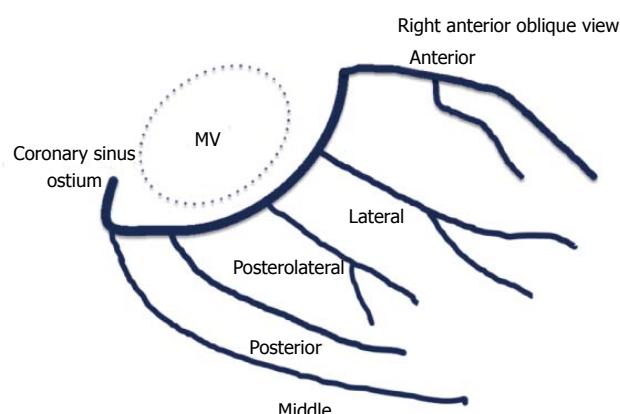


Figure 2 Coronary sinus anatomy as illustrated in the right anterior oblique view used to identify the optimal posterior, lateral and posterolateral branches for the left ventricular lead placement. MV: Mitral valve.

affect the efficacy of LV pacing may include the presence of transmural scar tissue and the degree of the mechanical contractile delay at the location where the LV lead is placed. It has been shown that the presence of LBBB leads to increased end-systolic volume and myocardial wall stress and decline of myocardial function^[4-7]. Early clinical data showed that LV pacing at the most delayed activated site reduces mechanical dyssynchrony and improves LVEF and LV remodeling^[8]. Over the last several years, with the use of modern echocardiographic techniques, such as tissue Doppler imaging, two dimensional strain and speckle tracking, a direct relationship between the improvement in NYHA class and the concordance of the placement of the LV lead tip in the maximally delayed activated LV site has been documented^[9-11]. Posterolateral and free wall LV pacing has been correlated with LV reverse remodeling defined by an increase of the ejection fraction and a decrease of the end-diastolic diameter. Butter *et al*^[12] examined the hemodynamic effects of different site LV pacing and they found that biventricular pacing, consisting of RV apex and LV free wall or anterior site pacing, was correlated with increased $+dP/dt_{max}$ values of LV, suggesting that lateral and posterior branches are the optimal LV pacing sites. Van Campen *et al*^[13] examined the effect of combined different pacing sites (RV apex or outflow tract and CS posterolateral or anterolateral) regarding the echocardiographic increase in cardiac index. They concluded that the CS posterolateral vein and RV apex configuration was the site with maximal increase in cardiac index in 29% of patients, the CS posterolateral and RV outflow tract (RVOT) combination produced the maximal increase in cardiac index in 21% of patients and CS anterolateral and RV apex in 19% of patients, respectively. This study suggested that the hemodynamic response and the increase in cardiac index varied between patients and moreover the changes were sustained over a 3-mo period^[13]. Earlier, Gold *et al*^[14] compared LV pacing from lateral and anterior sites and they reported no significant group differences in hemodynamic effects among different stimulation sites, although a larger hemodynamic effect with lateral wall stimulation was noted. A recently published study analyzed retrospectively

data from 457 recipients of CRT either with a pacemaker or with a defibrillator. Improvement in NYHA class was significantly greater in patients who underwent LV lead implantation in anterolateral and posterolateral sites with a tendency for greater improvement in LVEF in these regions compared to anterior wall. Long-term survival as estimated with the Kaplan-Meier method at 4 years varied by location (anterolateral: 72%, anterior: 48%, posterolateral: 62%, and posterior: 72% ($P = 0.003$))^[15].

Although the above data support the superiority of the lateral *vs* non-lateral LV lead placement sites, results from some of the major CRT trials perhaps report different outcomes. Thus, in the COMPANION trial, LV leads were located anteriorly (26%), posteriorly (10%) and the majority laterally (64%). Mortality rates in patients who received CRT with defibrillator were indifferent to LV lead position. Also, all functional outcomes, including 6-min walk distance, quality of life parameters and functional class, improved with CRT regardless of LV lead location^[16]. The REVERSE study indicated that a lateral LV wall pacing was beneficial concerning reverse LV remodelling and the composite of time to death or first HF hospitalization, while the position of the RV lead tip was indifferent^[17]. The PROSPECT study evaluated different LV pacing sites in three different groups of patients with evidence for CRT using a fluoroscopy-based clockwise principle: group A, “optimal” (between 3 and 5 o’clock and longitudinal basal/mid-position), group B, “non-optimal” (between 12 and 2 o’clock and longitudinal mid-apical anterior position) and group C (all other positions). No relation was found between the groups and CRT outcome or all-cause mortality. However, further sub-analyses, when groups A and C were combined *vs* B, suggested that the LV pacing site may impact outcomes in non-ischemic patients, those with LBBB, and when LV lead is located in an apical position^[18]. In MADIT-CRT trial, the LV lead location was classified with the use of coronary venograms and X-rays along the short and long axis into an anterior, lateral, or posterior region and basal, mid ventricular, or apical region, respectively. During the follow-up period, the primary end point (HF hospitalization or death) was similar for leads in the anterior, lateral or posterior sites, whereas patients with LV lead in the apical region exhibited a significantly increased risk of death or HF^[19].

IMPACT OF MYOCARDIAL SCAR BURDEN AND IMAGING ON CRT RESPONSE

The extent of myocardial scar has been inversely related to clinical response in patients undergoing a CRT device implantation. Although there are data supporting that there is no difference between patients with ischemic and non-ischemic cardiomyopathy concerning CRT response, plenty of studies have shown significant differences in the efficacy of CRT depending on the etiology of cardiomyopathy^[20,21]. The MIRACLE study revealed that CRT was more beneficial, with respect to LVEF and

reverse remodeling, in patients with non-ischemic HF and less severe mitral regurgitation. To assess the transmural extent of LV scar tissue, cardiac magnetic resonance imaging (MRI) with delay enhancement is currently the preferred imaging method of choice. Bleeker *et al*^[22] used contrast enhanced MRI to define LV scar burden and reported that those who failed to respond to CRT were more likely to have transmural scar in the posterolateral region of the LV. It is noteworthy that from 14 patients who had scar in the posterolateral wall, 11 had significant dyssynchrony as assessed by tissue doppler imaging (TDI) but a clinical benefit from CRT was only seen in 2 of these 11 patients.

The TARGET trial is the first randomized study that was designed to assess the impact of targeted LV lead placement, using baseline echocardiographic speckle-tracking 2-dimensional radial strain imaging *vs* conventional approach, on clinical CRT outcomes. After 6 mo, patients who underwent the echocardiography-guided implantation had a greater extent of LV reverse remodelling, better clinical response as well as lower HF hospitalization, although there was no difference in all-cause mortality. Multivariate analysis suggested that the greatest benefit was demonstrated in patients with a concordant LV lead at sites free of scar, whereas in patients with either an LV located remote to the latest site of contraction or in scar area, the response was significantly lower^[23]. More recently, results from the STARTER study, which randomized patients on echo-guided transvenous LV lead placement, determining the site of latest time to peak radial strain by speckle tracking echocardiography, *vs* a conventional fluoroscopy approach confirmed the superiority of the echocardiography-guided approach. Using intention-to-treat analysis, patients in the echocardiography group had a significant more favorable event-free survival (fewer HF hospitalizations or deaths) and furthermore, LV lead placement concordance with the site of latest mechanical activation was achieved significantly higher in these patients compared to others^[24]. The impact of LV lead position on LV dyssynchrony in CRT recipients was evaluated also by two-dimensional speckle tracking radial strain echocardiography in the study of Kristiansen *et al*^[25]. Mechanical dyssynchrony was assessed by anteroapical-to-posterior delay and interventricular mechanical delay and the LV lead was targeted to the latest activated LV segment (concordant). At 6-mo follow up, superior LV reverse remodeling, as defined by $\geq 15\%$ LV end-systolic volume reduction, was observed to be significantly higher in patients with concordant compared to those with discordant LV leads. Moreover, mechanical resynchronization responders 6 mo after CRT were significantly more in this group and the concordant LV lead was the only independent predictor of LV reverse remodeling^[25]. Gated single photon emission computed tomography (SPECT) has also been used to identify the scar region in CRT recipients. Ypenburg *et al*^[26] assessed the importance of transmural scar quantified by gated SPECT in the LV pacing target region and reported that transmural scar in the region of the LV pacing lead (as determined by chest X-ray) was negatively related to subsequent LV reverse remodelling although patients with transmural scar at the LV tip pacing site exhibited less LV dyssynchrony.

Table 1 Echocardiographic and non-echocardiographic methods for atrioventricular and interventricular optimization

AV optimization	VV optimization
Echocardiography-based methods	
Aortic and mitral VTI (velocity time integral)	Aortic VTI
3D echo	TDI
Device integrated methods	
Smart Delay™	QuickOpt™
QuickOpt™	AdaptiveCRT™
AdaptiveCRT™	Peak Endocardial Acceleration sensor
	SonR®
Peak Endocardial Acceleration sensor SonR®	
Other methods	
Acoustic cardiography	
Finger plethysmography	

AV: Atrioventricular; TDI: Tissue Doppler Imaging; VTI: Velocity-time integral; VV: Interventricular.

LV LEAD MULTISITE AND ENDOCARDIAL PACING

Transvenous LV lead implantation *via* CS cannulation is the currently adopted technique for CRT device implantation procedures. During the recent years, a new technique has been described, consisting of true multisite pacing with a second LV lead placed in a second branch of the CS, especially in patients with large LV dimensions in order to reduce the mechanical dyssynchrony. Leclercq *et al.*^[27] reported that triple site pacing was correlated with significantly better LV reverse remodeling, in terms of higher LVEF, and smaller LV end-systolic volume and diameter after 9-mo follow up. The recently published results of the TRUST CRT (“Triple Site Versus Standard Cardiac Resynchronization Therapy”) study indicated that after 12 mo of follow up significantly fewer patients with triple site CRT were in NYHA functional class III or IV compared to those with conventional CRT. Moreover, the incidence of serious, CRT-related adverse events was similar in triple-site and conventional group^[28].

Endocardial LV pacing theoretically offers greater options for pacing to patients in whom the CS system is inaccessible. There is always a need for lifelong anticoagulation therapy and the available clinical data although they are positive regarding efficacy and safety, they are still limited^[29,30]. LV endocardial pacing was compared to a single epicardial pacing site in the study of Padeletti *et al.*^[31] and the investigators reported no significant differences between endocardial and epicardial pacing configurations in terms of LV systolic or diastolic function measurements. Nevertheless, the optimal LV endocardial site producing the best LV function improvement was consistently better than the chosen epicardial pacing location.

OPTIMIZATION OF CRT DEVICE PROGRAMMING

The optimal CRT device programming is crucial in the

post implantation period in order to achieve the maximum percentage of biventricular pacing. Optimization of both AV and VV timing intervals have been suggested as potential methods to improve response rates and even increase the magnitude of symptomatic improvement in these patients. Nevertheless, data from multicenter trials in CRT recipients suggest that AV and VV optimization has limited efficacy on clinical outcomes and echocardiographic parameters, compared with a fixed 100-120 ms AV delay and simultaneous biventricular pacing^[32-36]. The combination of the optimal LV lead implantation site and optimal device programming is considered the gold standard for the best response in CRT recipients. However, the optimization of AV and VV delays in patients with non-optimal LV pacing site could partly ameliorate the hemodynamic effect^[37]. Several methods have been proposed to optimize the AV and VV delays and have been classified into two main groups: echocardiography-based and non-echocardiography-based methods (Table 1).

Echocardiography-based methods

The goal of AV optimization is to ensure that LV contraction does not occur before complete filling, whereas with VV optimization the goal is to minimize LV mechanical dyssynchrony. AV optimization is achieved using the Ritter method which aims at maximizing the LV filling during diastole by allowing for mitral valve closure to occur after a complete atrial systole. The interval between QRS onset and closure of mitral valve is measured by programming short and long AV intervals. It is noteworthy that the Doppler A wave is truncated with short AV delay programming and the opposite happens with very long AV delay which can cause fusion of the E and A waves and mitral valve diastolic regurgitation. The optimal AV delay is calculated as the long AV delay less the difference of the time intervals of the QRS onset to mitral valve closure at short and long AV intervals^[38].

AV optimization is also performed with the estimation of the maximal stroke volume measuring the aortic velocity-time integral (VTI) with multiple AV intervals^[39]. Similarly, the same measurements could be performed using diastolic mitral inflows including E and A waves. The measurement of the maximum mitral inflow VTI has been shown to correlate better with the maximal LV dP/dT values^[39]. In patients with mitral regurgitation, LV dP/dT can also be measured by the continuous Doppler curve of mitral regurgitation jet and determines better functional class and LVEF at 6-mo follow-up relative to an empiric AV delay program^[40]. New echocardiographic techniques, such as three dimensional echocardiography, are also used for CRT optimization leading to improvement of LVEF, stroke volume and myocardial performance index^[41]. AV delay optimization has been shown to have chronic beneficial hemodynamic effects, when it was performed 31 ± 8 wk after CRT device implantation and at a follow up period of 43 ± 5 d later. A slight significant increase in LVEF and 6-min walk time was reported and a significant decrease in N-terminal of the prohormone brain natriuretic peptide (NT-proBNP) values^[42].

Concerning VV optimization, the commonly used method is to calculate the maximal aortic VTI usually with pulse wave Doppler, which is considered to be a representative index of stroke volume^[33]. The time interval between LV and RV activation could be adjusted (commonly LV activation is usually preferred to precede) in the available CRT devices. TDI is also used to identify LV areas displaying delayed longitudinal contraction in order to achieve the optimum interventricular delay programming^[43]. There is no ideal method for AV and VV optimization as the results from clinical studies are controversial. A direct comparison of different echocardiographic measurements for VV interval optimization showed that aortic VTI and VV dyssynchrony were the most feasible (100% and 93% of feasibility, respectively)^[44]. On the contrary, Zuber *et al.*^[45] reported the superiority of acoustic cardiography derived electromechanical activation time compared to aortic VTI for AV and VV delay optimization.

Device integrated methods /automated algorithms

Automatic CRT optimization algorithms, based on intracardiac electrograms (IEGMs), have been developed to calculate the optimal AV and VV delays and consequently to improve the clinical outcomes. The Smart AV Delay™ (Boston Scientific Corporation, Minneapolis, MN, United States) algorithm estimates the sensed and paced AV intervals and the duration of native VV conduction time from the IEGMs and can only be used in patients with QRS duration ≥ 120 milliseconds, normal AV conduction, and intrinsic sensed or paced AV intervals from 100 milliseconds to 400 milliseconds. The algorithm aims at maximal resynchronization which is thought to occur when there is optimal fusion between intrinsic conduction through the interventricular septum and the paced activation of the late activated region of LV^[34]. The QuickOpt™ (St. Jude Medical, St. Paul, MN, United States) algorithm is based on the duration of intrinsic atrial depolarization, as measured from the right atrial electrogram duration, and determines the optimal sensed AV delay and ensures that ventricular pacing occurs after atrial depolarization and mechanical contraction are complete. The paced AV delay is always set as the optimal sensed AV delay plus 50 milliseconds. The QuickOpt™ software also includes calculation of optimal VV timing, measuring the interval for maximal intrinsic activation between the LV and RV leads and taking into account the VV conduction delay during both LV and RV pacing alone^[46]. The AdaptiveCRT™ (Medtronic, Minneapolis, MN, United States) algorithm uses electrograms to calculate the AV delay to optimize fusion of the LV-pacing-derived wavefront with that from intrinsic conduction. The algorithm provided mostly synchronized LV pacing and demonstrated better clinical outcomes compared to echocardiography-optimized biventricular pacing^[47]. The Peak Endocardial Acceleration sensor (SonR®, Sorin CRM SAS, Clamart, France) is embedded in the RV or atrial pacing electrode and determines the optimal AV and VV delays based on the peaks of

endocardial acceleration. Its effectiveness was evaluated in the multicenter CLEAR study which showed a significant improvement in subjective NYHA functional class, with the SonR® algorithm compared with usual practice^[36].

CLINICAL TRIALS RESULTS

CONCERNING AV AND VV

OPTIMIZATION

The Frequent Optimization Study Using the QuickOpt Method (FREEDOM) trial concluded that optimization using QuickOpt did not significantly influence outcome as defined by the HF clinical composite score^[46]. Additionally, the SMART-AV trial using Smart Delay™ algorithm reported that a fixed AV delay of 120 milliseconds was not inferior to the optimal AV delay, as derived from echocardiography or Smart Delay™ algorithm^[34]. Long term outcomes of VV interval optimization were investigated in the InSync III, RHYTHM II ICD and DECREASE-HF trials. The InSync III trial demonstrated that the optimal VV interval ranges between RV and LV pre-excitation of 40 milliseconds, respectively with a higher prevalence of LV pre-excitation although the sequential CRT optimization improved only the 6 min walking distance^[48]. Similarly, data from the RHYTHM II ICD study demonstrated no clinical benefit after 3-6 mo of follow-up by the optimized sequential CRT over the simultaneous biventricular pacing^[33]. Furthermore, the DECREASE-HF trial, which enrolled patients with QRS duration > 150 milliseconds and symptomatic HF, examined the potential benefits comparing simultaneous *vs* optimized biventricular pacing. Furthermore, at 6-mo follow-up, no significant differences between these two pacing modes (simultaneous *vs* optimized biventricular pacing) were reported regarding the reduction in LV size and the improvement of LVEF^[49].

CONCLUSION

CRT is an important treatment approach in selected patients with HF^[50]. The maximum desired results are achieved with the proper patient selection according to proposed indications, and with careful pre-implant and post-implant management. Although initial studies of different LV anatomic pacing sites suggested benefit of posterior or lateral sites, subsequent data has yielded conflicting results. Based on the current data, LV lead placement to sites of latest LV mechanical activation, as defined by speckle tracking echocardiography, remains a better method to improve the clinical results. Multisite and endocardial LV pacing are promising methods, but additional data are required. On the other hand, the role and efficacy of AV and VV optimization in improving clinical outcomes in CRT, albeit promising, remains unclear and there is no clearly superior technique or algorithm. It remains to investigate whether AV and VV interval optimization may improve the long-term survival.

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Complicated Whipple's disease and endocarditis following tumor necrosis factor inhibitors

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Abstract

AIM: To test whether treatment with tumor necrosis factor inhibitors (TNFI) is associated with complications of *Tropheryma whipplei* (*T. whipplei*) infection.

METHODS: Because unexplained arthritis is often the first Whipple's disease (WD) symptom, patients may undergo treatment with TNFI before diagnosis. This may influence the course of infection with *T. whipplei*, which causes WD, because host immune defects contribute to the pathogenesis of WD. A literature search and cross referencing identified 19 reports of TNFI treatment prior to WD diagnosis. This case-control study compared clinical data in patients receiving TNFI therapy (group I, $n = 41$) with patients not receiving TNFI therapy (group II, $n = 61$). Patients from large reviews served as controls (group III, $n = 1059$).

RESULTS: The rate of endocarditis in patient group I was significantly higher than in patient group II (12.2% in group I vs 1.6% in group II, $P < 0.05$), and group III (12.2% in group I vs 0.16% in group III, $P < 0.01$). Other, severe systemic or local WD complications such as pericarditis, fever or specific organ manifestations were increased also in group I as compared to the other patient groups. However, diarrhea and weight loss were somewhat less frequent in patient group I. WD is

typically diagnosed with duodenal biopsy and periodic acid Schiff (PAS) staining. PAS-stain as standard diagnostic test had a very high percentage of false negative results (diagnostic failure in 63.6% of cases) in group I. Polymerase chain reaction (PCR) for *T. whipplei* was more accurate than PAS-stainings (diagnostic accuracy, rate of true positive tests 90.9% for PCR vs 36.4% for PAS, $P < 0.01$).

CONCLUSION: TNFI trigger severe WD complications, particularly endocarditis, and lead to false-negative PAS-tests. In case of TNFI treatment failure, infection with *T. whipplei* should be considered.

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Key words: Arthritis; Complication; Endocarditis; Periodic acid-Schiff stain; Polymerase chain reaction; *Tropheryma whipplei*; Whipple's disease

Core tip: Arthritis frequently is the first symptom of Whipple's disease (WD). Therefore, many patients are treated with anti-inflammatory drugs or tumor necrosis alpha inhibitors (TNFI) before diagnosis. As host immune defects contribute to the pathogenesis of WD, immunosuppressive therapy may deteriorate the course of *Tropheryma whipplei* (*T. whipplei*) infection. In this study, it is shown that treatment with TNFI is associated with severe complications of *T. whipplei* infection, particularly with endocarditis. TNFI therapy may lead to false negative periodic acid-Schiff-tests and thereby hinder the diagnosis of WD. *T. whipplei* infection should be considered in case of TNFI treatment failure.

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INTRODUCTION

Tropheryma whipplei (*T. whipplei*) is an actinobacteria that may cause Whipple's disease (WD), a chronic and systemic infection. WD in its classical form mostly occurs in middle-aged Caucasian men and is clinically characterized by weight loss, diarrhea and arthritis. A broad range of other symptoms such as abdominal pain, melena, fever, cardiac symptoms, cough, lymphadenopathy and symptoms of the central nervous system (CNS) may be observed^[1,2].

Classical WD is very rare, although *T. whipplei* occurs ubiquitously in the environment. This discrepancy has been explained in part by cellular immune defects and a certain human leucocyte antigen type that predisposes individuals for infection^[3]. The genome of *T. whipplei* is very small, and shows some specific features such as a lack of thioredoxin pathway and a high variability of surface structures which point to a host dependency and a "parasitic" nature of the bacterium^[4]. Diagnosis of WD is usually established by duodenal biopsy and histological stain for periodic acid-Schiff (PAS), and/or a *T. whipplei* specific polymerase chain reaction (PCR)^[5].

Localized ("isolated") clinical forms of WD (*i.e.*, without gastrointestinal or systemic symptoms) may be manifestations of the CNS or endo-/pericarditis. These clinical manifestations are difficult to diagnose and are associated with a poor prognosis, and therefore require an intensive treatment and follow-up^[3,5].

It is well known that the first symptoms in patients with WD in approximately two-thirds of patients are seronegative, migratory and non-destructive arthropathies, which precede other symptoms by approximately 8 years^[1,5,6]. Many patients with arthropathies are treated with non-steroidal anti-inflammatory drugs (NSAIDs), or with other non-biological disease-modifying anti-rheumatic drugs (DMARDs) prior to the diagnosis of WD. It has been previously shown that intestinal manifestations (*i.e.*, diarrhea) of WD may be triggered by DMARDs^[6].

As treatment with NSAID or DMARDs lacks a prolonged clinical effect in patients with *T. whipplei* infection, patients may be subsequently treated with biological DMARDs, mostly with tumor necrosis factor alpha inhibitor (TNFI). Although TNFI are reasonable safe immunosuppressive drugs^[7], therapy with TNFI may be associated with an increased rate of infections, particularly with opportunistic infections and the activation of latent tuberculosis^[8-11]. We aimed to examine data on the clinical course and frequency of symptoms and complications in patients with WD who had received TNFI therapy prior to diagnosis compared to WD patients who had not received such treatment.

MATERIALS AND METHODS

For this case-control study, a literature search was performed with the following keywords in the PubMed and Cochrane databases in all combinations: Whipple, Whipple disease, Whipple's, Whipple's disease, intestinal

lipodystrophy, Tropheryma, *T. whipplei*, biological therapy, tumor necrosis factor alpha, TNF antagonist, TNF inhibitor, anti-TNF, TNF blocker, etanercept, infliximab, adalimumab, certolizumab, golimumab, immunosuppressant, immunosuppressive, and immunosuppression.

In total, 15 publications were identified in which WD patients were treated with TNFI^[12-26]. Another four unlisted references, mostly abstracts or non-English articles, could be tracked by cross-referencing^[27-30]. In several instances, the authors of the reports were contacted for more detailed information on the WD patients.

The patients in this case-control study were stratified according to their prior treatment and compared to large databases of reviews. In patient group I, WD patients ($n = 41$; 19 publications) were treated with non-biological DMARDs and with TNFI. Patient group II consists of WD patients ($n = 61$; same 19 publications) treated with non-biological DMARDs, but not with TNFI. Groups I and II were compared to WD patients from large reviews (patient group III, $n = 1059$)^[31-33]. One citation is a monography (696 patients)^[31], another review covers patients (238 patients) from this monography and presents some more details^[32], and one paper is a follow-up case analysis to the monography ($n = 363$)^[33]. In group III, few patients were treated with DMARDs (mostly steroids), but not with TNFI.

The clinical course of the patients were compared including major symptoms (arthralgia, weight loss, and diarrhea) and complications (such as fever, septic temperatures, endocarditis, pericarditis, immune-mediated symptoms, gastrointestinal complications, neurologic symptoms, skin manifestations, lymphadenopathy, and eye complications). Other less frequent symptoms could not be compared systematically due to the protean features of WD in many patients.

Statistical analysis

Statistical analysis of differences between patient groups and for the comparison of the PAS- and PCR-tests was performed with the Pearson's χ^2 test. Significance levels are expressed as two-sided *P* values. In parallel, the Fisher's exact test was performed which did not show different significance levels.

RESULTS

Forty-one patients were identified in whom TNFI were used to treat unexplained arthritis, and in whom the diagnosis of WD was established later (patient group I, Table 1). These patients received non-biological DMARDs prior or in parallel to therapy with TNFI.

When patients in group I were compared to patients in group II (non-biological DMARD therapy, but no therapy with TNFI), there was a highly significant increase in the rate of endocarditis ($P < 0.05$). Additionally, compared to patients from large literature reviews (group III), the percentage of patients with endocarditis in patients treated with TNFI was dramatically higher (50 times higher, 12.2% in patient group I vs 0.16% in

Table 1 Frequency of the symptoms at the time of diagnosis of Whipple's disease

	Patient group I	Patient group II	Patient group III
Patients (n)	41	61	1059
Therapy with TNFI	+	-	-
Therapy with non-biological DMARD	+	+	-
Signs or symptoms			
Arthritis	85.4%	88.5%	70%-90%
Fever	53.6%	44.3%	35%-60%
Weight loss	36.6%	42.6%	80%-96%
Diarrhea	35.3%	54.1%	70%-85%
Endocarditis	12.2%	1.6% ^b	0.16% ^b
Pericarditis	12.2%	3.3%	0.08% ^b
IRIS	16%	22.9%	0%

All patients treated with non-biological disease modifying anti-rheumatic drugs (DMARDs) or tumor necrosis factor alpha inhibitor (TNFI) had experienced arthropathy or arthritis before the start of the therapy. In patient group III, few patients were treated with non-biological DMARDs (mostly steroids), but none were treated with TNFI. In 16 patients (reference 22), some symptoms (neurologic or eye symptoms) are not explicitly stated. The percentage of neurologic or eye symptoms of the analyzed patients were comparable (20% in group I ($n = 25$), 21.3% in group II ($n = 45$), and 10%-25% in group III). ^b $P < 0.01$ vs group I. IRIS: Immune reconstitution inflammatory syndrome.

patient group III, $P < 0.01$). Additionally, pericarditis in patient group I was more frequent than in patient group III ($P < 0.01$). Pericarditis had an inflammatory course in all reported patient courses.

The rate of patients with diarrhea in group I was lower than in group II, and less than half in group III. Additionally, and as a possible consequence of the reduced rate of diarrhea, weight loss was less frequent in group I than in other patients. The immune reconstitution inflammatory syndrome (IRIS), which mostly occurs after medical immunosuppression^[14-16], was observed in comparable percentages in the patients of groups I and II (16% and 22.9%, respectively). The remaining signs or symptoms of patients treated with TNFI (patient group I) were similar to the other patient groups (Table 1). The details of group I patients (19 publications reporting 41 individuals) are given in Table 2.

In 63% of group I patients severe systemic or local complications were observed (1.88 complications per patient, Table 3). Very often, fever or septicemia occurred. In addition to endocarditis, pericarditis or IRIS, a broad range of complications, e.g., spondylitis, colitis, lymphadenopathy, and CNS symptoms was observed. The outcome of the patients treated with TNFI (group I) was lethal in one patient (out of 19 patients with reported outcome), and two other patients had severe long-term sequelae (Table 3).

Despite TNFI therapy, arthralgia persisted or clinical deterioration developed within months in many patients in group I. This often led to intensified diagnostic procedures, especially to endoscopy with duodenal biopsies. Subsequent PAS staining or PCR tests finally led to the diagnosis of WD. When analyzing the accuracy

of diagnostic tests for patients who were diagnosed and treated with WD, there was a very high rate of false-negative PAS tests. Therefore, only 8/22 PAS tests (36.4%) either before or after TNFI therapy were true positive. Conversely, 14 out of 22 PAS stains (63.6%) were negative in patients for whom WD was diagnosed by other means. In contrast, 10 out of 11 PCR tests were positive for the diagnosis of WD before or after the initiation of TNFI therapy (Tables 4 and 5). Therefore, PAS stainings were significantly more unreliable than PCR-testing. Taken together, PCR for *T. whipplei* was much better as compared to PAS stain (diagnostic accuracy, rate of true positive tests 90.9% for PCR vs 36.4% for PAS, $P < 0.01$).

DISCUSSION

In this case-control study we demonstrate that the risk of severe *T. whipplei*-associated complications, particularly the rate of endocarditis, is increased by TNFI therapy in patients with a later diagnosis of WD. This observation is of interest for several reasons. First, endocarditis with *T. whipplei* is one of the major reasons for serious or lethal outcomes of WD, and requires prolonged and intensive antibiotic treatment regimens^[1,3,34]. Second, *T. whipplei* seems to have a certain tropism for heart valves. Endocarditis with *T. whipplei* - a bacterium that occurs ubiquitously in the environment - has been described to be the fourth most frequent cause of culture-negative endocarditis^[35]. With the availability of *T. whipplei* specific PCR, a higher diagnostic frequency of WD endocarditis in the future seems probable^[31,32,36]. Third, the risk of bacterial endocarditis may be increased somewhat by TNFI therapy in general, because certain genetic haplotypes in the tumor necrosis factor gene (single nucleotide polymorphisms) predispose patients for bloodstream infections or endocarditis^[37], and signals of increased rates of endocarditis after TNFI therapy have been observed in a treatment registry^[38]. Therefore, in patients with endocarditis under biological DMARD therapy, the possibility of TNFI therapy-associated *T. whipplei* endocarditis should be considered, and the rate of endocarditis following TNFI should be monitored in patient series.

Infectious complications including opportunistic infections may occur after therapy with TNFI, which disturbs the important host defense mechanisms^[10]. Latent tuberculosis can be activated, because TNFI breach the cellular integrity, disturb granuloma formation, and viable mycobacteria in granulomas become released^[39]. Therefore, it is mandatory to exclude tuberculosis before initiating therapy with TNFI. As another example for activation of infection, TNFI therapy reduces interferon (IFN)-gamma levels which may lead to salmonella septicemia^[40].

T. whipplei is an ubiquitously occurring bacterium that can be detected via stool PCR, e.g., in asymptomatic carriers (4%, healthy population), in sewage plant workers (20%), and in relatives of WD patients (38%)^[1,41,42]. It causes acute infections such as fever and diarrhea in

Table 2 Summary of cases treated with tumor necrosis factor alpha inhibitor and later diagnosis of Whipple's disease

Patients (n) (sex, age, yr)	TNFI/time from treatment start of TNFI to diagnosis of WD	Other DMARDs	Clinical picture at the time of diagnosis of WD (after TNFI treatment)	Therapy for WD/outcome	Ref.
1 (F, 33)	Etanercept; 6 mo	NSAID, MTX	Chronic inflammation, ankylosing spondylitis, arthritis, diarrhea, fever, weight loss	CFA + CTM; resolution	[12]
1 (M, 57)	Etanercept; "mo"	Leflunomide, MTX	Endocarditis, aortic valve replacement	DCN, HCQ; resolution	[13]
1 (M)	Etanercept; not stated	Steroids, others not specified	Arthralgia, diarrhea, others not specified, IRIS after Abx	Long-term steroids, resolution	[14]
1 (M, 70)	Etanercept; 19 mo	NSAID, sulfasalazine, MTX	Ankylosing sacroiliitis, endocarditis, dyspnea, fever, aortic valve replacement	DCN, CTM, HCQ; resolution	[15]
3 (M, 51-76)	Infliximab/2, etanercept/2 "yr"	Steroids, MTX, gold, leflunomide, cyclophos- phamide, cyclosporine	IRIS after Abx, fever, 3 arthritis, pseudotumor orbitae	3 long-term steroids; 1 blindness; 1 jejunal perforation; 1 death after 3 yr	[16]
1 (M, 46)	Infliximab, adalimumab; 28 mo	MTX, steroids	Ankylosing sacroiliitis, fever, T. whipplei septicemia, diarrhea	CFA + CTM; resolution	[17]
1 (M, 35)	Etanercept; 2 mo	None	Purpura, scurvy, diarrhea, pancolitis, granulomas, T. whipplei septicemia, arthritis	CTM; resolution	[18]
5 (4 M, 1 F, 38-67)	Etanercept/4, infliximab/2, adalimumab/2; 2, 4, 9, 26, 85 mo	NSAID, MTX, abatacept, rituximab, chloroquine, steroids, leflunomide	Aortic endocarditis, 4 arthritis, 3 T. whipplei in blood or septicaemia, 3 weight loss, 2 diarrhea, 2 pericarditis, 2 meningitis, diarrhea, hemorrhagic colitis, lymphadenopathy	1 DCN 4 DCN + HCQ All with favorable outcome	[19]
1 (M, 34)	Infliximab; 0, 5 mo	NSAID, steroids, azathioprine, MTX	Erythema nodosum, diarrhea, weight loss, fever, sigmoido-vesical fistula, arthritis, lymphadenopathy	CTM; resolution	[20]
1 (M, 47)	Adalimumab; "mo"	MTX, steroids, leflunomide	Arthritis, recurrent fever, cough, weight loss	CFA + CTM; resolution	[21]
16	TNFI not specified; time not stated	Most had other immuno- suppressants	Immunosuppressants: 43% deterioration, 32% temporary relief, then deterioration, 25% ineffectiveness; TNFI: 2 patients with endocarditis	Not specified	[22]
1 (M, 62)	Etanercept; "short"	NSAID, steroids, MTX	Polyarthritis, pericarditis, cutaneous lesions; T. whipplei septicaemia	Penicillin G, CTM; persisting cutaneous lesions; DCN + HCQ; resolution	[27]
1 (F, 58)	Not specified; 24 mo	No data available	Arthritis	No data available	[28]
1 (M, 53)	Not specified; 12 mo	No data available	Pericarditis, polyarthritis, lower limb edema	No data available	[29]
1 (M, 67)	Etanercept; "short"	MTX, steroids, sulfasalazine, leflunomide	Pericarditis, pulmonary hypertension, arthritis, candida esophagitis, segmental ulcerative intestinal lesions, episcleritis, fever	CFA + CTM; Stopped because of diarrhea; Cefixim, steroids, resolution	[23]
1	Etanercept; not stated	No data available	No data available	No data available	[30]
2 (M, 42, 53)	Etanercept/2, adalimumab/2; 13, 14 mo	NSAID	Deterioration of arthritis, spondylising arthritis, diarrhea, weight loss	1 DCN + HCQ, Sulfadiazin; 1 DCN + HCQ; 2 resolution	[24]
1 (M, 64)	Etanercept; 6 mo	Steroids, MTX	Spondylising arthritis, multisegmental spondylitis, arthritis, weight loss, Giardia lamblia infection	CFA + CTM; Persistent inflamma-tion, then DCN +HCQ; resolution	[25]
1 (M, 52)	Etanercept, adalimumab, infliximab; 24 mo	Steroids, MTX	Rash, hemiplegia, dysarthria, facial palsy, cognitive sequelae, headache, decrease of executive functions, arthritis, diarrhea, weight loss, lymphadenopathy	CFA + CTM, amelio-ration, but remaining cognitive sequelae, headache	[26]

CFA: Ceftriaxone; CTM: Cotrimoxazole; DCN: Doxycycline; F: Female; HCQ: Hydroxychloroquine; IRIS: Immune reconstitution inflammatory syndrome; M: Male; MTX: Methotrexate; NSAID: Non-steroidal anti-inflammatory drugs; steroids: Corticosteroids; TNFI: Tumor necrosis factor alpha inhibitor; WD: Whipple's disease; DMARD: Disease modifying anti-rheumatic drug; T. whipplei: Tropheryma whipplei.

Table 3 Whipple's disease related symptoms and outcome after therapy with tumor necrosis factor alpha inhibitor

Sign or symptom ¹	Patients (n)
Total number of severe complications	49 (1.88 complications per patient)
Total number of affected patients	26 out of 41 patients (63%)
Fever, septic temperatures	16
<i>T. whipplei</i> septicaemia, <i>T. whipplei</i> in blood	6
Pericarditis	5
Endocarditis (many with valve replacement)	5
IRIS	4
Spondylitis (multisegmental)	4
Colitis, fistula, perforation	4
Neurologic symptoms, meningitis	3
Severe skin manifestation	3
Lymphadenopathy	2
Severe eye complications, blindness	2
Ankylosing sacroiliitis	2
Pulmonary hypertension	1
Outcome	
Severe sequelae or death	3/19
Blindness	1
Neurological symptoms (cognitive sequelae, dysfunction)	1
Death	1
Resolution or favorable outcome	16/19
Not specified/no data	22

¹Many patients experienced arthritis, weight loss or diarrhea as specified in Table 2. IRIS: Immune reconstitution inflammatory syndrome; WD: Whipple's disease; *T. whipplei*: *Tropheryma whipplei*.

children, occurs as localized infection (e.g., endocarditis or in the CNS), or causes the rare, classical WD^[1,3,43]. The hallmarks of classical WD are the long-term persistence of the viable bacteria in duodenal macrophages, and reduced T helper cell 1 responses in patients^[5,44,45]. In analogy to latent tuberculosis, TNFI therapy may disturb the balanced immunological control, impair IFN- γ further, and subsequently lead to an activation or deterioration of the systemic (e.g., sepsis, IRIS) or local (e.g., endocarditis) *T. whipplei* infection. Our data therefore provide further hints to the opportunistic nature of *T. whipplei* from the clinical point of view. Our data also expand the pathogenetic understanding and concept of *T. whipplei* infection, because it remained unclear why the wide-spread, low-pathogenic organism would infrequently lead to clinical manifestations, i.e., to systemic WD. With respect to our results, immunosuppression plays a role in the progression of asymptomatic *T. whipplei* infection to chronic and systemic WD.

The observations in this study also require attention because WD diagnosis by duodenal biopsies with PAS stain is unreliable in TNFI pretreated patients. Therefore, in this situation diagnosis should be based on PCR, and screening strategies in the future may apply stool PCR^[46] before and during therapy with TNFI.

The reason that PAS-staining is apparently much less frequently diagnostically accurate under biological DMARD therapy is unclear. Additionally, it remains unclear why the percentage of common clinical WD

Table 4 Lack of reliability of standard diagnostic procedures in patient group I: Different situations ("settings") of the diagnostic procedures

Setting	Diagnostic procedure for suspected WD before TNFI therapy	Diagnostic procedure for suspected WD after TNFI therapy	Patients (n) (reference)
1	Duodenal biopsy PAS neg	Duodenal biopsy PAS neg PCR pos	3 [19,21,25]
2	Duodenal biopsy PAS neg	Duodenal/small bowel biopsy PAS pos (PCR not performed)	3 [19,20,25]
3	"Duodenal mucosa normal"	Duodenal/jejunal PAS pos PCR pos	1 [23]
4	Duodenal biopsy PAS neg	Explanted heart valve PCR pos	1 [13]
5	Duodenal biopsy PAS neg PCR neg	Explanted heart valve PCR pos	1 [15]
6	None performed	Duodenal biopsy PAS pos PCR pos	3 [17,24,26]
7	None performed	Duodenal biopsy PAS neg PCR pos	2 [12,27]
8	None performed	Colonic biopsy PAS pos Stool, blood, CSF PCR pos	1 [18]
Total	9/9 PAS negative or mucosa normal 1/1 PCR negative	5/13 intestinal biopsies PAS neg 8/13 intestinal biopsies PAS pos 10/10 intestinal PCR pos	15 patients, 13 reports

PAS: Periodic acid-Schiff staining; PCR: Polymerase chain reaction; TNFI: Tumor necrosis factor alpha inhibitor; WD: Whipple's disease. Details are not stated in 23 patients (references 14, 16, 22, 28-30). neg: Negative; pos: Positive.

Table 5 Lack of reliability of standard diagnostic procedures in patient group I: Summary of all diagnostic tests n (%)

	PAS-stain	PCR-test
	False negative	False negative
Before TNFI therapy	9/9 (100)	1/1 (100)
After TNFI therapy	5/13 (38.4)	0/10 (0)
Total	14/22 (63.6) ^b	1/11 (9.1)
	True positive	True positive
Before TNFI therapy	0/9 (0)	0/1 (0)
After TNFI therapy	8/13 (61.6)	10/10 (100)
Total	8/22 (36.4) ^b	10/11 (90.9)

^bP < 0.01 vs periodic acid-Schiff staining (PAS)- and polymerase chain reaction (PCR)-tests. TNFI: Tumor necrosis factor alpha inhibitor.

symptoms such as diarrhea and (subsequent) weight loss following TNFI therapy is lower, and the severe systemic complications of WD are more frequent. These observations could be due to the suppression of gastrointestinal involvement of *T. whipplei* and an induction of the systemic spread of the bacteria by TNFI treatment. These open questions should be clarified in future investigations which could also target the limitations of this study, i.e., the retrospective data analysis in a rare disorder.

In conclusion, our study shows that TNFI therapy may activate latent or asymptomatic infection with *T. whipplei*, leading to an aggressive course of WD and high rate of endocarditis. Therefore, clinical practice before

TNFI therapy should be modified, so that *T. whipplei* is excluded before therapy with TNFI in patients with unexplained arthritis. Moreover, if TNFI treatment failure or paradoxical therapy effects are observed, the possibility of *T. whipplei* infection should be considered. These safety concerns should be monitored in TNFI therapy registers.

COMMENTS

Background

Whipple's disease (WD) is a rare systemic infection with *Tropheryma whipplei* (*T. whipplei*), and host immune defects contribute to the pathogenesis. WD is typically diagnosed by duodenal biopsy and periodic acid-Schiff (PAS) stain. As unexplained arthritis is often the first WD symptom, patients may receive treatment with anti-inflammatory drugs or tumor necrosis alpha inhibitors (TNFI) before diagnosis. It remained unclear whether TNFI therapy is associated with a more complicated course of *T. whipplei* infection.

Innovations and breakthroughs

This study shows that treatment with TNFI may trigger severe complications of *T. whipplei* infection, particularly endocarditis. Medical immuno-suppression plays a role in the progression of asymptomatic *T. whipplei* infection to chronic and systemic WD. TNFI therapy may also lead to false-negative PAS-tests in WD and thereby hinder the diagnosis. In this situation, polymerase chain reaction has an important diagnostic role.

Applications

In case of treatment failure or paradoxical effects of TNFI treatment in patients with unclear arthritis, infection with *T. whipplei* should be considered.

Peer review

This is certainly an interesting and potentially important retrospective case control studies that had been investigated by the authors to look at the potential negative impact of using biological immunosuppression of arthritis patients without checking them for an undiagnosed Whipple's disease.

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Dangerous triplet: Polycystic ovary syndrome, oral contraceptives and Kounis syndrome

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Core tip: The young lady in our case has had suffered from hyperandrogenism, oligomenorrhea, polycystic ovaries and while was receiving oral contraceptives she developed intermittent angina attacks not strictly related to her medication. The angina attacks associated with increased cardiac enzymes increased high sensitivity cardiac troponin, skin itching and electrocardiographic changes suggesting of myocardial ischemia. Eosinophils were raised but the coronary arteries were normal. The angina attacks disappeared with discontinuation of contraceptives. Such angina attacks associated with such clinical setting are attributed to disease itself, to oral contraceptives and/or to Kounis hypersensitivity coronary syndrome, namely a dangerous triplet.

Erol N, Karaagac AT, Kounis NG. Dangerous triplet: Polycystic ovary syndrome, oral contraceptives and Kounis syndrome. *World J Cardiol* 2014; 6(12): 1285-1289 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i12/1285.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i12.1285>

Abstract

Polycystic ovary syndrome is characterized by ovulatory dysfunction, androgen excess and polycystic ovaries and is associated with hypertension, diabetes, metabolic syndrome and cardiovascular events. Oral contraceptives constitute first-line treatment, particularly when symptomatic hyperandrogenism is present. However, these drugs are associated with cardiovascular events and hypersensitivity reactions that pose problem in differential diagnosis and therapy. We present a 14 year-old female with polycystic ovary syndrome taking oral contraceptive and suffering from recurrent coronary ischemic attacks with increased eosinophils, and troponin levels suggesting Kounis syndrome.

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Key words: Contraceptives; Eosinophils; Kounis syndrome; Polycystic ovary syndrome; Troponin

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a clinical disorder characterized by ovulatory dysfunction, androgen excess and polycystic ovaries. It is the most common endocrine disorder in the females of reproductive age, mostly presented with obesity, glucose intolerance, hyperinsulinemia, dyslipidemia and hypertension. The females with PCOS carry an increased risk of cardiovascular ischemic events independent of the obesity^[1-3] with the prevalence of 4%-10%. In addition, the rate of occurrence of cardiovascular events in the 25-34-year-old female group with PCOS has been found to be 2.6%. Furthermore, therapy with combined oral contraceptives has been associated with both deep vein thrombosis and pulmonary embolism^[4] as well as hypersensitivity reactions^[5] that pose problems in differential diagnosis and treatment. We report a young

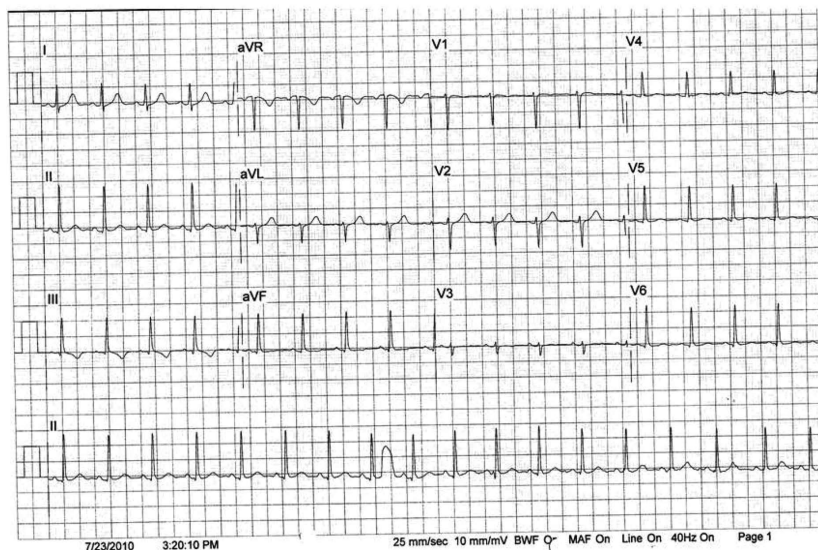


Figure 1 Electrocardiogram during chest pain with increased troponin showing T wave inversion in lead III and flattening of T wave in lead left foot derivation in electrocardiography.

14-year-old female patient with PCOS who was exposed to the above dangerous triplet and suffered from recurrent attacks of chest pain with high troponin levels and increased eosinophils while the coronary arteries were normal.

CASE REPORT

A 14-year-old female [56 kg, 160 cm, body mass index (BMI): 21.87 kg/m²] attended the endocrinology outpatient clinic for PCOS due to hyperandrogenism, oligomenorrhea, and polycystic ovaries. She was referred to our pediatric cardiology clinic for recurrent chest pain attacks and the elevated cardiac enzymes while she was receiving intermittent contraceptive medication.

In her past medical history, the prenatal and perinatal periods were uneventful. There was no consanguinity between her mother and father. There was no cardiovascular disease or any other chronic disease history in her family. Her physical growth and psychomotor development were compatible with her age. However, her thelarche and genital hair growth started at the age of 7.5 years. This was not accompanied by any menstrual bleeding. The family brought her to endocrinology outpatient clinic where laboratory investigation showed high testosterone and cholesterol levels. Additional laboratory investigation for adrenal pathology revealed no abnormality. She was advised not to take any medication up to 14 years of age. At this age endocrinologists decided to put her on oral contraceptive (ethinyl estradiol + 0.03 mg drospirenone 3 mg combination) which resulted in menstrual bleeding. However, she could not use contraceptives regularly due to nausea, headache and loss of appetite. Three months later and while she was receiving intermittently the contraceptive therapy she experienced severe chest discomfort with skin itching lasting for 15 min and she was transferred to the emergency department. Serum creatine kinase muscle was 57 U/L (normal levels: 0-24) and the troponin level was 11.91 ng/mL (normal levels: 0-0.04). Her pulse was 100 beats per minute regular and electrocardiogram showed sinus rhythm, T wave inversion in lead III and flattening of

T wave in lead left foot derivation in electrocardiography (Figure 1). Total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol levels were found to be 230 mg/dL, 290 mg/dL, 63 mg/dL, 108 mg/dL and 58 mg/dL, respectively. Eosinophils were also raised to 700/mm³ (normal levels up to 500/mm³). Chest radiography and echocardiography were also normal. Stress test, holter electrocardiogram and coronary computed tomography angiogram revealed no abnormality (Figure 2). The same clinical symptoms appeared while she was taking intermittently the contraceptive treatment and necessitating repeated hospital admissions, in approximately 1 or 2 mo intervals. At every hospitalization, repeated high sensitivity troponin levels and eosinophils were always elevated while the girl was feeling itchy. In view of the above findings we decided to change contraceptives to progesterone 5 mg and spironolactone 50 mg. After cessation of the oral contraceptive treatment, chest pain attacks disappeared despite taking the new medication. Biochemical tests, electrocardiogram (Figure 3), echocardiography, holter and repeated stress tests during the follow-up period of one year were all within normal limits. Cardiac enzymes and troponin returned to normal levels but eosinophils were still high (700/mm³). We did not perform skin prick tests and patch tests to oral contraceptives on ethical grounds. She was characterized as an atopic individual and was advised to refrain the previous contraceptive medication.

DISCUSSION

The diagnosis of PCOS is difficult because of the heterogeneity of the phenotype. The classical phenotype is observed in 75% of PCOS cases and cardiovascular risk factors are most frequently encountered in this group. Because several features of PCOS may be in evolution in adolescents, it was suggested recently^[6] that only firm criteria should be used to make a diagnosis of PCOS during adolescence. These criteria include hyperandrogenism,

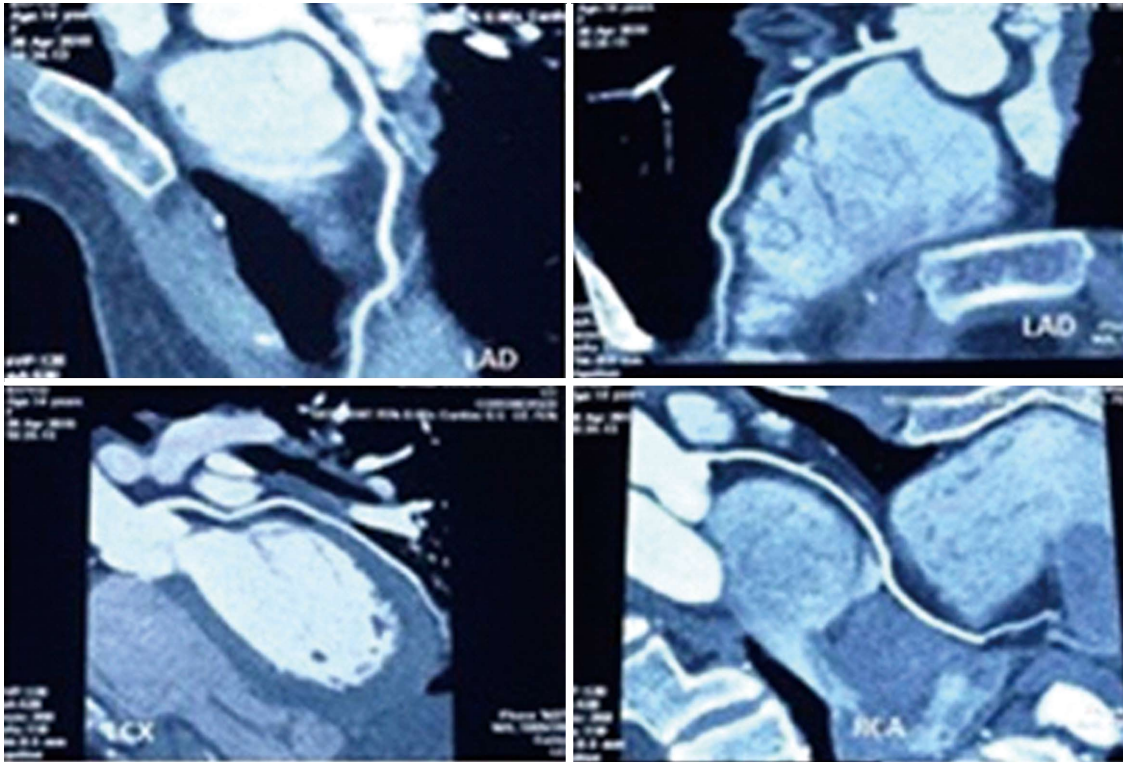


Figure 2 Normal computed tomography angiogram excluding thrombosis.

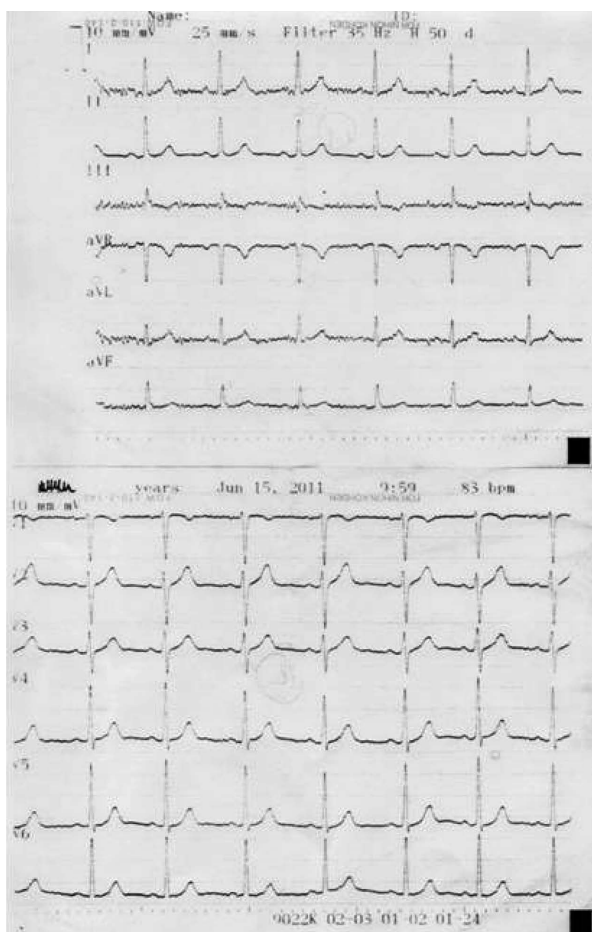


Figure 3 Normal electrocardiogram during cessation of contraceptive while the eosinophils were increased.

oligomenorrhea, and polycystic ovaries. Significant associations between PCOS and severity of cardiovascular diseases, family history of myocardial infarction as well as with elevated levels of insulin and triglycerides and lower levels of HDL-C have been reported^[7]. Metabolic syndrome, type 2 diabetes, abdominal obesity and hypertension, are frequently observed in young patients with PCOS, which are factors associated with cardiovascular diseases. Our patient has had dyslipidemia with increased cholesterol and triglycerides. Oral contraceptives are used for the treatment of PCOS. Combined oral contraceptives containing drospirenone are preferred especially in the PCOS patients with hirsutism^[8]. The risk of myocardial infarction and intracerebral events is 2.5% higher in the patients taking combined oral contraceptives containing low dose estrogen^[9]. Our patient had no findings suggesting drug-related venous thromboembolism but she had chest discomfort associated with increased high sensitivity troponin levels and eosinophilia that suggested coronary event. Drug-related Kounis syndrome has been reported on several occasions^[10]. In general, Kounis syndrome has an acute onset, but some subclinical cases have been reported^[11]. Several mediators such as, cytokines and chemokines including histamine, neutral proteases, arachidonic acid products and platelet activating factor are released during the allergic episode. The same mediators are also involved in the acute coronary syndromes. Several drugs have been accused for triggering this syndrome. However, the only drug our patient took was oral contraceptive consisting of ethinyl estradiol and drospirenone. Allergy to oral contraceptive therapy has been already described on several occasions. Erythema multiforme limited to the oral mucosa was reported in

a teenager on oral ethinyl estradiol and drospirenone therapy^[5]. Acquired angioedema has been also related to patients receiving oestrogen contraceptives and is frequently associated with recurrent urticarial^[12]. Allergic contact dermatitis has been reported in some occasions with transdermal therapeutic systems containing ethinyl estradiol^[13]. In view of the increased eosinophils, skin itching, normal coronary angiography, electrocardiographic changes and troponin increase diagnosis of type I variant of Kounis syndrome^[14] was made and the culprit medication was discontinued. Eosinophils have emerged as a novel biomarker of risk stratification in patients who have experienced coronary artery episodes^[15]. With cessation of combination of ethinyl estradiol and drospirenone and changing it to progestogen-only contraceptives, symptoms of the patient disappeared while eosinophil count was still increased denoting atopic diathesis. In women with PCOS and multiple cardiovascular risk factors the use of progestogen-only contraceptives is associated with substantially less risk of cardiovascular events but the danger is still watching for, since this drug can induce hypersensitivity reactions^[16].

Kounis syndrome, polycystic ovary syndrome and oral contraceptives seem to constitute a dangerous triplet and should be always suspected in order to establish correct diagnosis, apply appropriate treatment and avoid untoward consequences.

COMMENTS

Case characteristics

The young lady in this case has had polycystic ovary syndrome and while was receiving oral contraceptives she developed intermittent angina attacks, increased cardiac enzymes, increased high sensitivity cardiac troponin, eosinophilia with skin itching and electrocardiographic changes suggesting of myocardial ischemia.

Clinical diagnosis

Myocardial ischemia associated with Kounis syndrome, polycystic ovary syndrome, oral contraceptive therapy.

Differential diagnosis

Angina attacks with increased cardiac enzymes, increased high sensitivity cardiac troponin, electrocardiographic changes suggesting of myocardial ischemia and eosinophilia with normal coronary arteries in polycystic ovary syndrome are attributed to disease itself, to oral contraceptives and/or to Kounis hypersensitivity-associated coronary syndrome.

Laboratory diagnosis

Myocardial ischemia in polycystic ovary syndrome associated with oral contraceptive therapy and eosinophilia.

Imaging diagnosis

Coronary computed tomography angiogram performed upon the suspicion of thrombosis revealed normal coronary arteries.

Treatment

Discontinuation of contraceptive treatment and changing it to progesterone (medroxyprogesterone acetate) and spironolactone alleviated the symptomatology.

Related reports

Hyperandrogenism, oligomenorrhea, polycystic ovaries constitute the polycystic ovary syndrome which occasionally is associated with cardiovascular events, enhanced by hypersensitivity reaction to concomitant contraceptive medication.

Experiences and lessons

Patients with polycystic ovary syndrome who have to use oral contraceptives should be evaluated separately in terms of drug related cardiac events and hypersensitivity associated with Kounis syndrome.

Peer review

This case report is well written and addresses common problems with diagnostics of polycystic ovary syndrome associated with cardiovascular symptomatology, oral contraceptives and drug hypersensitivity culminating in the development of Kounis syndrome.

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Spontaneous coronary artery dissection as a cause of myocardial infarction

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Core tip: In this case report, we discussed a patient who had a rare disease called spontaneous coronary artery dissection simultaneously in two different coronary arteries causing acute myocardial infarction. The presence of muscular bridge and spontaneous coronary artery dissection together at the same site of the left anterior descending artery was another interesting point which made us report this case.

Aksakal A, Arslan U, Yaman M, Urumdaş M, Ateş AH. Spontaneous coronary artery dissection as a cause of myocardial infarction. *World J Cardiol* 2014; 6(12): 1290-1292 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i12/1290.htm>
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Abstract

Spontaneous coronary artery dissection (SCAD) is a rare disease that is usually seen in young women in left descending coronary artery and result in events like sudden cardiac death and acute myocardial infarction. A 70-year-old man was admitted to the emergency department with chest pain which started 1 h ago during a relative's funeral. The initial electrocardiography demonstrated 2 mm ST-segment depression in leads V1-V3 and the patient underwent emergent coronary angiography. SCAD simultaneously in two different coronary arteries [left anterior descending (LAD) artery and left circumflex (LCx)] artery was detected and SCAD in LCx artery was causing total occlusion which resulted in acute myocardial infarction. Successful stenting was performed thereafter for both lesions. In addition to the existence of SCAD simultaneously in two different coronary arteries, the presence of muscular bridge and SCAD together at the same site of the LAD artery was another interesting point which made us report this case.

INTRODUCTION

Spontaneous coronary artery dissection (SCAD) is a rare cause of acute coronary events or sudden cardiac death. It typically affects young healthy women, particularly in the peripartum period^[1]. SCAD has been reported in patients with collagen disease, cocaine abuse, severe hypertension and severe psychological stress^[2]. Herein, we reported a case of simultaneous dissection of two coronary arteries, one of which caused acute myocardial infarction.

CASE REPORT

A 70-year-old man was admitted to the emergency department with chest pain which started 1 h ago during a relative's funeral. The patient had abandoned smoking 10 years ago and had no history of any cardiac disease. Physical examination was unremarkable and the heart rate of 60 beats/min and the blood pressure of 90/60 mmHg

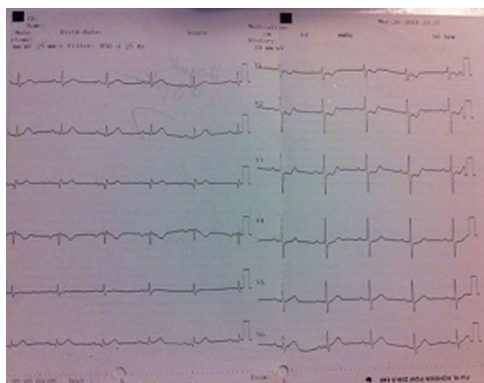


Figure 1 Electrocardiogram showing ST depression in leads V1-V3.

in the emergency unit. The initial electrocardiography demonstrated 2 mm ST-segment depression in leads V1-V3 (Figure 1). Bedside transthoracic echocardiography showed posterior hypokinesis with a left ventricle ejection fraction of 50%. After patient's transfer to coronary angiography laboratory, antiplatelet drugs, *i.e.*, 300 mg aspirin and 600 mg clopidogrel were administered. The patient underwent coronary angiography in which left circumflex artery (LCx) was totally occluded (Figure 2A) and left anterior descending artery (LAD) had a mid-segment dissection where a muscular bridge was located (Figure 2B). The LCx total occlusion was crossed with a floppy guidewire and then pre-dilated with balloon angioplasty. Then it was observed that LCx lesion had also dissection (Figure 2C). After careful evaluation of the previous angiographic views, the proximal segment of the LCx artery just proximal to the total occlusion had also dissection (Figure 2A), so it was thought that SCAD in LCx artery caused acute myocardial infarction. TIMI-3 blood flow was succeeded after stent implantation (3.0 mm \times 28 mm everolimus eluting Xience Pro[®] coronary stent) with a good angiographic result (Figure 2D). However, the patient's chest pain increased and blood pressure decreased. Left coronary angiogram at anteroposterior projection with cranial angulation revealed LAD mid-segment was sub-totally occluded (Figure 2E). This lesion where a muscular bridge and coronary dissection were found together, was thought to be aggravated after intracoronary nitrate infusion. Then it was successfully stented with a 3.0 mm \times 38 mm everolimus eluting coronary stent (Xience Pro[®]). TIMI-3 antegrade flow of LAD distal to the muscular bridge segment without any evidence of dissection was provided (Figure 2F). His hospital course was uneventful and he was discharged with medical treatment including aspirin, clopidogrel, metoprolol and atorvastatin. The patient was asymptomatic at 1-year clinical visit.

DISCUSSION

Primary SCAD is a rare cause of acute myocardial infarction and is with a high mortality rate of about 50%^[3]. The incidence of spontaneous coronary artery dissection for the public with AMI is estimated to be less than 1%^[4]. Due to the increased shear stress and proximities with the chest, it is

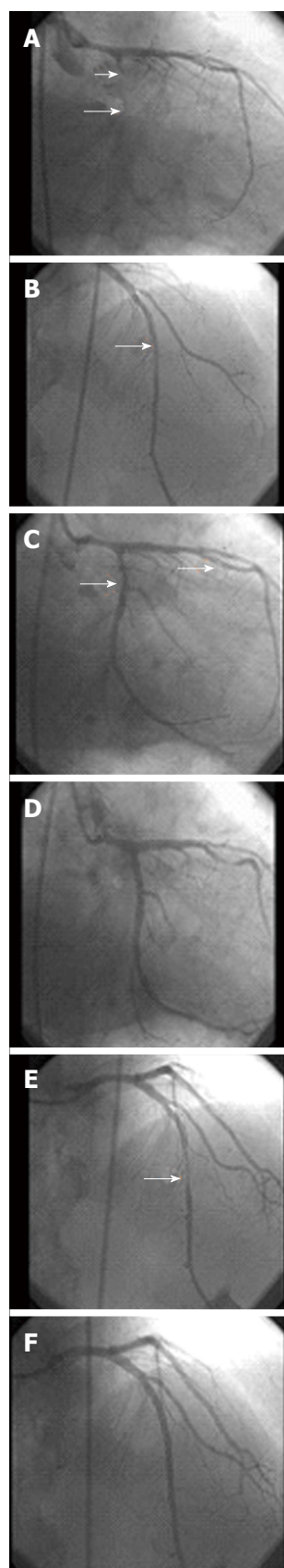


Figure 2 Coronary angiographic views of the patient. The arrows indicate left circumflex (LCx) total occlusion and proximal dissection in (A), coronary dissection in left anterior descending (LAD) (B), simultaneous coronary dissection in LAD and LCx arteries in (C) and subtotal occlusion and dissection at the site of muscular bridge in LAD in (E). Figure (D) and (F) show LCx and LAD arteries after successful stent implantation.

probable that dissection is widely found in the LAD. The spectrum of clinical presentation can range from unstable angina and myocardial infarction to sudden death. Early diagnosis and an aggressive treatment could improve the prognosis of patient with SCAD^[5,6]. Unfortunately there is no good definition of the optimal management of SCAD. The decision depends on the clinical presentation, hemodynamic condition, extent of the dissection, and number of vessels included^[7,8].

In our case, it was interesting that two coronary arteries had dissection at the same time. Despite the fact that the cause of LCx dissection might be balloon angioplasty of the total occlusion, we thought that spontaneous dissection was the cause of myocardial infarction because it was present just proximal to the total occlusion in LCx artery before the guidewire passage. An interesting point of this case was simultaneous dissection of the LAD and LCx arteries because multi-vessel SCAD is a rare situation discussed in few case reports^[9,10] and of them majority occurred in the peripartum period^[9]. In our case, the cause of the SCAD of both arteries might be the emotional stress that the patient came across.

In this case, the severity of LAD lesion was aggravated after intracoronary nitrate infusion during LCx artery stenting because the nitrates are known to increase the severity of lesions where muscular bridge is located. Another interesting point was the presence of the SCAD at the site of LAD where the muscular bridge was located. Whether the presence of muscular bridge facilitated the development of SCAD is not known but to our knowledge, the co-existence of these two different entities has not been published in the literature till now, so we cannot speculate such a cause-effect relationship.

In conclusion, we reported a rare case of SCAD simultaneously in two different coronary arteries causing acute myocardial infarction. The presence of muscular bridge and SCAD together at the same site of the LAD was another interesting point which made us report this case.

COMMENTS

Case characteristics

This case reported a case of simultaneous dissection of two coronary arteries, one of which caused acute myocardial infarction.

Clinical diagnosis

Physical examination was unremarkable and the initial ECG demonstrated 2 mm ST-segment depression in leads V1-V3.

Differential diagnosis

Acute coronary syndrome, pulmonary embolism.

Imaging diagnosis

The patient underwent coronary angiography in which left circumflex artery (LCx) was totally occluded and left anterior descending artery (LAD) had a

mid-segment dissection where a muscular bridge was located. After careful evaluation of the angiographic views, the proximal segment of the LCx artery just proximal to the total occlusion had also dissection.

Treatment

Stent implantation was performed to both lesions.

Related reports

It was rare in the literature that two coronary arteries had dissection at the same time. The presence of muscular bridge and spontaneous coronary artery dissection (SCAD) together at the same site of the LAD was another interesting point which has never been reported till now.

Experiences and lessons

Despite the rarity of SCAD, it may be the cause of acute coronary syndrome and cases should be evaluated separately to find the optimum treatment strategy.

Peer review

The authors have performed a good study, the manuscript is interesting.

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- 3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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- 4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

- 5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

- 6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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- 7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

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- 8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

- 9 Outreach: Bringing HIV-positive individuals into care. *HRS-A Careaction* 2002; 1-6 [PMID: 12154804]

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- 10 **Sherlock S**, Dooley J. Diseases of the liver and biliary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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- 11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

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- 12 **Breedlove GK**, Schorfeide AM. Adolescent pregnancy. 2nd ed. Wiczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

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- 13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

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- 14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

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- 15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: <http://www.cdc.gov/ncidod/eid/index.htm>

Patent (list all authors)

- 16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee.

Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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Write as mean \pm SD or mean \pm SE.

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