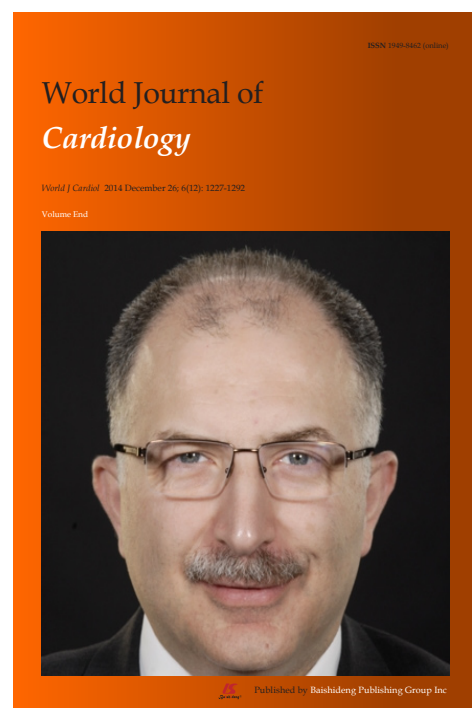
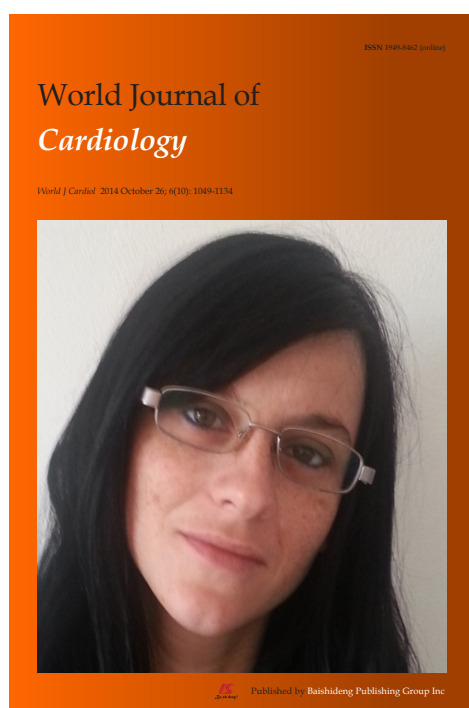
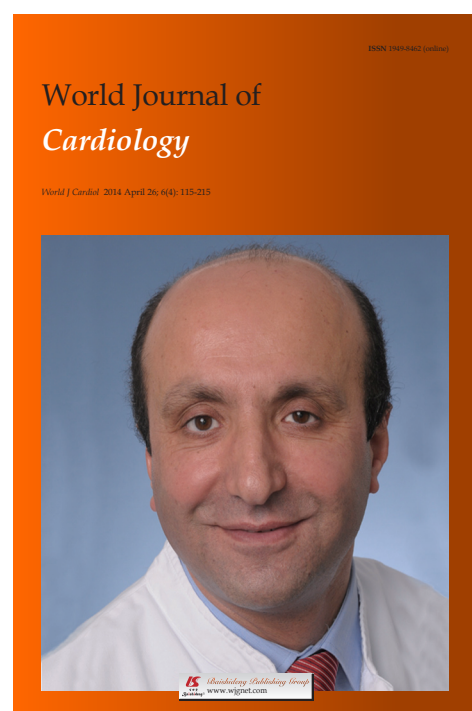
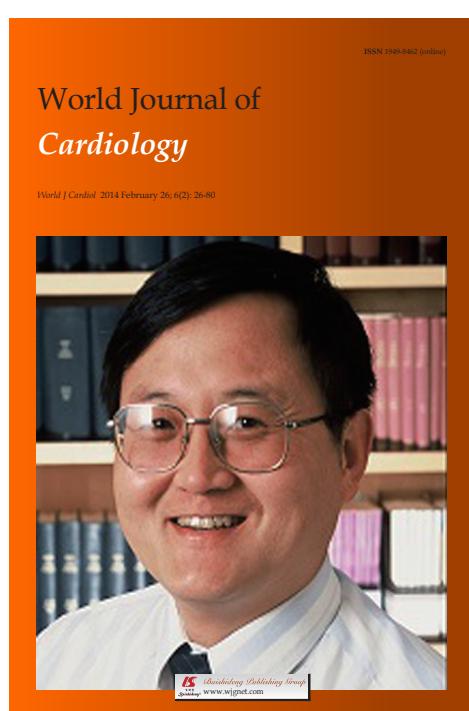


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World J Cardiol 2014 January 26; 6(1): 1-25





FIELD OF VISION

- 1 Percutaneous closure of patent foramen ovale: "Closed" door after the last randomized trials?
Hernandez J, Moreno R

MINIREVIEWS

- 4 Physiology of natriuretic peptides: The volume overload hypothesis revisited
Arjamaa O
- 8 Is diabetic cardiomyopathy a specific entity?
Letonja M, Petrovič D

BRIEF ARTICLE

- 14 Primary reperfusion in acute right ventricular infarction: An observational study
Lupi-Herrera E, González-Pacheco H, Juárez-Herrera Ú, Espinola-Zavaleta N, Chuquiure-Valenzuela E, Villavicencio-Fernández R, Peña-Duque MA, Ban-Hayashi E, Férrez-Santander S

LETTERS TO THE EDITOR

- 23 Occurrence of longitudinal stent compression before stent deployment: Two case studies
Aminian A, Lalmand J, Dolatabadi D

Contents

World Journal of Cardiology
Volume 6 Number 1 January 26, 2014

APPENDIX I-V Instructions to authors

ABOUT COVER Editor-in-Chief Member of *World Journal of Cardiology*, Giuseppe De Luca, PhD, Assistant Professor, Department of Cardiology, Piedmont University, Novara 28100, Italy

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Percutaneous closure of patent foramen ovale: "Closed" door after the last randomized trials?

Joel Hernandez, Raul Moreno

Joel Hernandez, Raul Moreno, Department of Interventional Cardiology, University Hospital La Paz, 28045 Madrid, Spain

Author contributions: Hernandez J wrote the manuscript; Moreno R provided the idea, insight, statistical analysis and manuscript edition.

Correspondence to: Raul Moreno, MD, PhD, FESC, Department of Interventional Cardiology, University Hospital La Paz, Paseo La Castellana 261, 28045 Madrid, Spain. raulmorenog@hotmail.com

Telephone: +34-68-7483054 Fax: +34-68-7483054

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Abstract

Patent foramen ovale (PFO) percutaneous closure has previously been an accepted intervention for the prevention of recurrent cryptogenic stroke on the basis of observational studies. However, randomized trials have been lacking until now. Three recently published randomized trials (CLOSURE I, PC and RESPECT) do not demonstrate the superiority of this intervention versus optimal medical therapy, therefore making this practice questionable. Nonetheless, these trials have had certain pitfalls, mainly a lower than initially estimated number of patients recruited, therefore lacking sufficient statistical power. On the other hand, different closure devices were used in the three trials. In two of them (PC and RESPECT), the Amplatzer PFO Occluder was used and the STARflex device was used in the other one (CLOSURE I). Taken altogether, a meta-analysis of these three trials does not demonstrate a statistically significant benefit of percutaneous PFO closure (1.9% vs 2.9%; $P = 0.11$). However, if we analyze only the PC and RESPECT trials together, in which the Amplatzer PFO Occluder was used, a statistically significant benefit of percutaneous PFO closure is observed (1.4% vs 3.0%, $P = 0.04$). In conclusion, our interpretation of these trials is that the use of a dedicated, specifically designed Amplatzer PFO device could possibly reduce

the risk of stroke in patients with PFO and cryptogenic stroke. This consideration equally applies to patients who have no contraindications for anticoagulant or antithrombotic therapy.

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Key words: Patent; Foramen; Ovale; Closure; Percutaneous; Device; Cryptogenic; Stroke; Risk

Core tip: Percutaneous patent foramen ovale (PFO) closure has been used for the prevention of recurrent cryptogenic stroke on the basis of observational studies; however, recent randomized trials do not support its use for this indication. A detailed analysis of these randomized trials could suggest that when the Amplatzer PFO Occluder is used, the risk of stroke is reduced.

Hernandez J, Moreno R. Percutaneous closure of patent foramen ovale: "Closed" door after the last randomized trials? *World J Cardiol* 2014; 6(1): 1-3 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i1/1.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i1.1>

COMMENTARY ON HOT TOPICS

Patent foramen ovale (PFO) is present in a very high proportion of healthy subjects but as its frequency is higher in patients that have suffered a cryptogenic stroke, PFO has been accepted as a potential cause of stroke, especially in younger patients and in the presence of atrial septal aneurysm^[1-3]. As a result, percutaneous closure of PFO has been performed in some patients that have suffered a cryptogenic stroke and in whom a PFO has been demonstrated. The indications of this procedure have been widely debated. Guidelines have been conservative, accepting this strategy only for patients with recurrent

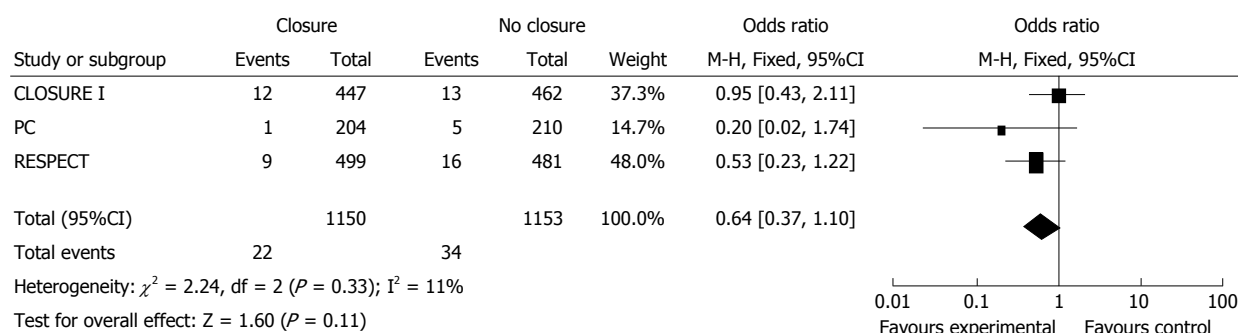


Figure 1 Meta-analysis of all three randomized trials.

stroke despite antithrombotic therapy^[4], but this procedure has also been performed in many patients after a first stroke, mainly in younger patients and in those with a concomitant atrial septal aneurysm.

Non-randomized studies suggested that the recurrence of stroke in patients with cryptogenic stroke was lower if a percutaneous closure of PFO was performed, compared with patients that remained on medical therapy alone^[2,5,6]. However, the main limitation for a wider acceptance of percutaneous closure has been the absence of randomized trials^[4].

Last year, the final results of the CLOSURE I trial were published. In this study, 909 patients between 18 and 60 years of age with a cryptogenic stroke (72%) or transient ischemic attack (TIA) (28%) and a PFO were randomized to percutaneous closure using the STARflex (NMT Medical Inc.) device in addition to medical treatment (aspirin 81 or 325 mg daily for two years and clopidogrel for the first six months) or to medical treatment alone (aspirin 325 mg daily and/or warfarin for a target INR 2.0-3.0) and followed-up for two years^[7]. This study was negative, since the primary endpoint at 2 years (stroke or TIA, death from any cause during the first 30 d, or death from neurological causes between 31 d and 2 years) was not reduced with percutaneous closure (5.5% *vs* 6.8% in the medical therapy group; $P = 0.37$). Moreover, the risk of stroke at 2 years was similar between both groups of patients (2.9% with percutaneous closure *vs* 3.1% with medical treatment; $P = 0.79$). The CLOSURE I had some limitations, such as a much lower than initially intended number of patients recruited (909 instead of 1600)^[8], patients with either stroke or TIA were included, three of twelve (25%) strokes occurred within 30 d after the procedure, other possible causes of stroke became apparent in patients who had recurrences, patients with prothrombotic disorders were excluded, and randomization was not locally blind. Another possible explanation for the negative results is the relatively short follow-up period^[9].

Nonetheless, these results were very discouraging, especially for interventional cardiologists. On top of this, two other negative randomized trials regarding the same issue but using a device specifically designed for PFO closure (Amplatzer PFO Occluder, St Jude Medical) have been published in March of this year^[10,11]. The RESPECT trial^[10] randomized 980 patients to medical treatment or PFO closure using the Amplatzer PFO Occluder. The

primary endpoint was the occurrence of recurrent ischemic stroke or early death in patients 18-60 years of age. The intention-to-treat analysis was negative (HR = 0.49, 95%CI: 0.22-1.11, $P = 0.08$), but due to a high dropout rate in the medical treatment group, the between-group difference was significant in the rate of recurrent stroke in the pre-specified per-protocol cohort (HR = 0.37, 95%CI: 0.14-0.96, $P = 0.03$) and in the as-treated cohort (HR = 0.27, 95%CI: 0.10-0.75, $P = 0.007$).

The PC trial randomized patients with a PFO and ischemic stroke, TIA or a peripheral thromboembolic event to undergo closure of the PFO with the Amplatzer PFO Occluder or to receive medical therapy. The primary endpoint was a composite of death, nonfatal stroke, TIA or peripheral embolism and was not reduced with percutaneous closure (HR = 0.63, 95%CI: 0.24-1.62, $P = 0.34$). Non-fatal stroke occurred in 1 patient (0.5%) in the closure group and 5 patients (2.4%) in the medical therapy group (HR = 0.20, 95%CI: 0.02-1.72, $P = 0.14$).

A simplistic interpretation of these three trials could lead us to conclude definitively that percutaneous closure of PFO is not effective in reducing the risk of stroke in patients with cryptogenic stroke. Since these trials have been flawed by marked difficulties in patient recruitment, it is evident that each of them individually will probably lack sufficient power to prove any possible differences. In this sense, if we perform a pooled analysis from the 3 trials, including 2303 patients overall, percutaneous closure of PFO does not reduce the incidence of stroke (1.9% *vs* 2.9%, $P = 0.11$; Figure 1). However, if we include only the 2 trials in which an Amplatzer PFO Occluder device, specifically designed for PFO, was used, percutaneous closure was associated with a significant reduction in the incidence of stroke (1.4% *vs* 3.0% $P = 0.04$; Figure 2).

Possible explanations for these differences may be the following: the STARflex closure system has been associated with a significantly higher thrombosis rate at 30 d than the Amplatzer PFO Occluder device in two different studies, 3.6% *vs* 0%, $P < 0.01$ and 5.7% *vs* 0%, $P < 0.05$ ^[12,13], and the incidence of atrial fibrillation^[14] has also been documented more frequently at 30 d with STARflex (4.5% *vs* 1.3%; $P = 0.02$). Also, a lower rate of periprocedural complications in the PC and respect trials could partly explain the better results of percutaneous closure in the PC and RESPECT trials.

Our interpretation of these trials is that the use of a dedicated, specifically designed Amplatzer PFO device

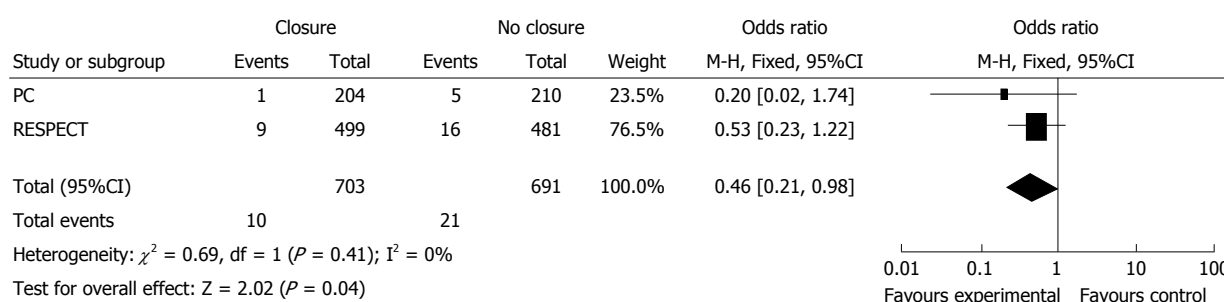


Figure 2 Meta-analysis of the two trials using an Amplatzer Patent Foramen Ovale Occluder.

could possibly reduce the risk of stroke in patients with PFO and cryptogenic stroke. Therefore, although present evidence does not support PFO closure for the prevention of recurrent cryptogenic stroke, a detailed analysis of recent randomized trials can make us consider that the door for PFO closure might not be entirely closed. This consideration equally applies to patients who have no contraindications for anticoagulant or antithrombotic therapy.

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Physiology of natriuretic peptides: The volume overload hypothesis revisited

Olli Arjamaa

Olli Arjamaa, Department of Biology, University of Turku, 20014 Turku, Finland

Author contributions: Arjamaa O solely contributed to this paper. Correspondence to: Olli Arjamaa, MD, PhD, Senior Scientist, Department of Biology, University of Turku, Vesilinnamäki, 20014 Turku, Finland. olli.arjamaa@utu.fi

Telephone: +358-4-05125452

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Abstract

The discovery of the natriuretic peptide system in the early 1980s aroused great interest among clinical cardiologists. The heart was not a mechanical pump alone, but also an endocrine organ that had powerful effects on blood circulation. Natriuretic peptides caused both natriuresis and diuresis, and they responded to a volume overload which caused either stretch or pressure on the heart. As a result, the findings led to the conclusion that the human body had a hormone with effects similar to those of a drug which treats high blood pressure. Later, it became evident that the volume contraction was fortified by extrarenal plasma shift. Here, a hypothesis is presented in which the role of natriuretic peptides is to regulate oxygen transport as the volume contraction leads to hemoconcentration with an increased oxygen-carrying capacity. Wall stress, either chemical or mechanical, changes the oxygen gradient of the myocardium and affects the diffusion of oxygen within a myocyte. In support of this hypothesis, hypoxia-response elements have been found in both the atrial natriuretic peptide and the brain natriuretic peptide genes.

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Key words: Natriuretic peptides; Hypoxia; Hemoglobin concentration; Volume overload

Core tip: A new concept is suggested for the understanding of the physiology of natriuretic peptides. Both chemical and physical challenges will ultimately increase the oxygen consumption of the heart which is the factor regulating the release of natriuretic peptides. Diuresis, natriuresis and plasma shift lead to hemoconcentration and the oxygen transport in human body will be enhanced.

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INTRODUCTION

In a recent state-of-the-art review, Mangiafico *et al*^[1] discuss the possibility of inhibiting the natriuretic peptide system by neutral endopeptidases as an evolving strategy to treat hypertension and heart failure. The concept behind this review and the related drug trials, such as in the case of Solomon *et al*^[2], has been that both atrial natriuretic peptide [ANP (A-type)] and brain natriuretic peptide [BNP (B-type)] are secreted from the heart as a result of direct wall stress, caused either by stretch or pressure affecting cardiocytes, to protect the human body from a volume overload. NT-proBNP especially, the biologically inactive sequence of proBNP with a long half-time and circulating in blood, has been utilized either as an indicator of the metabolism of natriuretic peptides or as a guide of treatment in a wide array of heart diseases. The hypothesis was formulated about thirty years ago when a large and rapid intravascular volume increase resulted in high plasma levels of ANP in rats^[3] and since then has prevailed without an alternative interpretation. At that time, it was also shown that an infusion of rat heart atrial extracts into a rat's circulation brought about massive diuresis and natriuresis^[4], reaffirming the hypothesis.

These findings were greeted with excitement in cardiology; now we had an endogenous hormone available that could combat all pressure-caused heart diseases, similar to those of the drugs previously developed to treat high blood pressure. The large numbers of articles published on natriuretic peptides, more than 28000 by the end of 2013, reflect the high expectations in clinical cardiology towards these peptides over a broad time frame, but perhaps also that the physiological role of the natriuretic peptide system in healthy humans has not been definitely clarified. As a result, the significance of the natriuretic peptide as a tool in cardiology has remained obscure.

PHYSIOLOGY OF NATRIURETIC PEPTIDES

The conclusion that a direct mechanical load on myocytes is the key factor regulating the synthesis and release of the natriuretic peptide system, occurring across the whole animal kingdom, is rather confusing as it bypasses the function of nervous stretch receptors in the atria and disregards the effects of variable flow conditions in the atrial lumen occurring during physical activity. In addition, terrestrial mammals living in a dry and warm environment do not experience large intravascular volume overloads but, on the contrary, are constantly in danger of becoming dehydrated. In his review on volume and pressure regulation, Guyton, the single author of several textbooks of medical physiology and a specialist in blood pressure regulation, did not refer to the natriuretic peptide system as a pressure controller at all, a role which he gave solely to the kidneys^[5]. What was not known in the early 1980s and became evident later, was that a natriuretic peptide has strong extrarenal vascular actions, contributing to contracting the plasma volume by transferring fluid and plasma protein from plasma to interstitial compartments^[6].

Apart from the pharmacological interest in developing a new class of drugs to treat high blood pressure, based on the volume overload hypothesis, Baertschi *et al*^[7] showed that hypoxia was a direct and sufficient stimulus for ANP release from an isolated rodent heart. Later, hypoxia-sensitive elements were found from the promoter sequence of both the *ANP* and the *BNP* genes^[8,9]. In line with these findings, there are many studies, performed with isolated myocytes, heart muscle strips and animals, which clearly provide evidence that there is a hypoxia sensitive component in the release mechanism of the natriuretic peptide system. When the blood flow in the coronaries of the pig heart was surgically blocked, the BNP mRNA increased significantly in the wall area that had become hypoxic^[10] and the plasma levels of NT-proBNP were associated with the extent of myocardial damage and microvascular obstruction in patients, as assessed by contrast-enhanced cardiac magnetic resonance imaging^[11]. Stockmann *et al*^[12] studied the effects of oxygenation in the hypertrophied heart ventricular of the rat and showed that when normoxic conditions were restored, the ANP content decreased to control levels despite the persisting

hypertrophy. Salmon cardiac peptide, a hormone related to A-, B- and C-type natriuretic peptides^[13] and localized in salmon heart ventricle^[14], has a hypoxia sensitive component in its release mechanism which is independent of contraction^[15].

It is interesting to note that in the clinical studies in which the oxygen delivery into contracting myocytes is impaired, the measurement of natriuretic peptides has shown its strength. A meta-analysis of 2784 patients from sixteen studies identified stress-induced myocardial ischemia as a significant condition linked with high plasma levels of BNP^[16]. In a five year prospective longitudinal clinical study with 4775 primary care subjects, a single measurement of NT-proBNP significantly improved the prediction of incident cardiovascular events^[17]. The combined endpoint in this study was restricted to the occurrence of myocardial infarction, coronary revascularization and cardiovascular mortality due to a sudden cardiac death or a fatal myocardial infarction. When comparing troponin assays with NT-proBNP assay in an acute coronary syndrome, Gravning *et al*^[18] showed that the latter assay was superior to former ones to predict the long-term mortality in a prospective study of 458 patients. NT-proBNP predicted the extent of coronary artery disease and ischemia in the patients with stable angina pectoris, thus contributing to the diagnostic process^[19], and was linked to the severity of the aortic valve disease^[20]. The recent ACTION Registry-GWTG study^[21] reports the measurements of natriuretic peptides from a cohort of almost 30000 patients admitted to hospitals with an acute myocardial infarction. Among these patients without heart failure, natriuretic peptides were strongly and independently associated with the in-hospital mortality, even after adjustments for the severity of presentation. Also, in the patients with paroxysmal, persistent atrial fibrillation, most probably causing elevated oxygen consumption, plasma levels of natriuretic peptides were increased^[22]. This accumulating experimental and clinical evidence for a direct role for oxygen has, however, been overshadowed by the wall stress hypothesis which alone has been used as a magnifying glass when looking at clinical results.

CRITICAL DEBATE

What if the wall stress hypothesis has been misleading clinical cardiologists for nearly thirty years? The volume overload hypothesis originates from a rather small number of physiological experiments made in the 1980s. Additionally, as the following decade saw the rundown of physiology departments due to the strong emergence of molecular biology, the focus of natriuretic peptide research was rapidly moved towards clinical applications.

The role of the natriuretic peptide system is perhaps not to counterbalance pressure changes in circulation, but to regulate oxygen transport, both locally and systemically, by causing volume contraction (diuresis, natriuresis and *plasma shift*) leading to hemoconcentration and an increased oxygen-carrying capacity per unit volume of blood^[23-25]. All the conditions that will increase the oxygen consumption or change the oxygen diffusion of

Table 1 Established facts

Already known fact 1	Volume overload (stretch or pressure) stimulates the synthesis and release of natriuretic peptides
Already known fact 2	Natriuretic peptides cause natriuresis, diuresis, vasodilatation and plasma shift

myocytes, such as stretch, pressure or metabolic challenges, will ultimately initiate the synthesis and enhance the release of natriuretic peptides from intracellular locations. Although these conclusions can be partly deduced from existing experimental and clinical results, more precise evidence can be obtained with the following sophisticated methods to support cardiologists in reanalyzing and reinterpreting their previous findings and to bring the natriuretic peptide associated drug development back onto a biologically correct basis.

To initiate a paradigm shift, the following methods should be introduced for the studies of the pathophysiology of natriuretic peptides. A method that is able to reveal perfusion defects in patients suffering from ischemia is positron emission tomography. Although the method has been available for several years, the properties of the tracers used have limited the interpretation of results. By means of newer tracers with a better defect contrast than the previous ones, it is or will be possible to quantify the perfusion of the myocardium during an exercise test or under a pharmacological challenge in patients with ischemia^[26].

Further evidence on the role of oxygenation can be obtained during congenital heart surgery with open chest cavity when an optical probe can be placed directly onto the free wall of the right ventricle, measuring the myoglobin saturation of myocytes^[27].

Experimentally, the Langendorff perfusion system is the method of choice if the effects of hypoxic conditions on the natriuretic peptide system are to be studied *in vitro*^[28]. The isolated rodent heart can be perfused with different types of buffer solution, containing molecules with oxygen-carrying capacity, under appropriate left ventricular preload and afterload pressures. Imaging the fluorescence of NADH (the reduced form of nicotinamide adenine dinucleotide) from a local hypoxic ventricular area provides a measure of the mitochondrial redox state and the method has revealed that in the isolated biventricular working rabbit heart, different pacing rates produce hypoxic conditions^[29]. In addition, the gene targeting technology of natriuretic peptides may provide us with new insights into their diverse functions and especially into the role of hypoxia in the physiology of natriuretic peptides^[30]. Even the assessing the oxidative metabolism of a single myocyte with NADH fluorescence is possible^[31].

In all the methods mentioned above, natriuretic peptides can be measured either from the circulating plasma or from the perfusate and the concentration can be compared with the state of tissue oxygenation.

Table 2 Novel insights

New information 1	Natriuretic peptide system responds to oxygen tension (hypoxia-response elements in the promoter sequence of ANP and BNP genes). Volume overload causes wall stress and changes the consumption or diffusion of oxygen in heart
New information 2	The result of natriuresis, diuresis and plasma shift is volume contraction and increased oxygen-carrying capacity per unit volume of blood. Oxygen transport will be enhanced

ANP: Atrial natriuretic peptide; BNP: Brain natriuretic peptide.

NEW CONCEPT

According to the hypothesis outlined here, any chemical or mechanical challenge directed towards myocytes will eventually affect the diffusion or consumption of oxygen within a myocyte^[32], producing functional and regional heterogeneity of the oxygen supply-consumption ratio in the heart. During large and rapid changes in wall tension, as have occurred in volume overload experiments *in vivo* and pressure increase experiments with the Langendorff preparation *in vitro*, these manipulations have necessarily affected the oxygen metabolism of the heart. Interpreting the results from studies with single myocytes, isolated perfused hearts and with patients suffering from ischemia from a new angle will provide us with a new concept of the physiology of the natriuretic peptide system in healthy humans. To sum up, the role of the natriuretic peptide system is to increase oxygen transport in healthy humans to counteract hypoxic conditions and the stimulus to which the synthesis and release of natriuretic peptides responds is the oxygen gradient among cardiocytes (Tables 1 and 2).

It is worth noting that, in seal pups, able to experience a physiological eupnea-apnea cycle while sleeping, the plasma ANP was significantly higher when they were holding their breath than during the periods of eupnea^[33]. Also, blood from seals showed an increase in hematocrit from 55.6% to 63.1% with a peak occurring within 1 min of the end of apnea^[34], reflecting an increased hemoglobin concentration.

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Is diabetic cardiomyopathy a specific entity?

Mitja Letonja, Danijel Petrovič

Mitja Letonja, Department of Internal Medicine, General Hospital Ptuj, 2250 Ptuj, Slovenia

Danijel Petrovič, Institute of Histology and Embryology, Medical faculty, University Ljubljana, 1000 Ljubljana, Slovenia

Author contributions: Letonja M and Petrovič D designed review paper and wrote the paper.

Correspondence to: Danijel Petrovič, MD, PhD, Professor, Institute of Histology and Embryology, Medical Faculty, University Ljubljana, Vrazov trg 2, 1000 Ljubljana, Slovenia. daniel.petrovic@mf.uni-lj.si

Telephone: +386-1-5437360 Fax: +386-1-5437367

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Abstract

Diabetes mellitus (DM) is characterised by hyperglycemia, insulin resistance and metabolic dysregulation leading to diastolic and systolic dysfunction in diabetes. In this review, the pathogenetic and pathomorphological changes leading to diastolic and systolic dysfunction in diabetes are discussed. Changes in metabolic signalling pathways, mediators and effectors contribute to the pathogenesis of cardiac dysfunction in DM called diabetic cardiomyopathy (DC). Echocardiographic studies report on the association between DM and the presence of cardiac hypertrophy and myocardial stiffness that lead to diastolic dysfunction. More recently reported echocardiographic studies with more sensitive techniques, such as strain analysis, also observed systolic dysfunction as an early marker of DC. Depression of systolic and diastolic function is continuum and the line of separation is artificial. To conclude, according to current knowledge, DC is expected to be a common single phenotype that is caused by different pathogenetic and pathomorphological changes leading to diastolic and systolic dysfunction in diabetes.

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Key words: Diabetes mellitus; Diabetic cardiomyopathy; Pathogenesis; Diastolic dysfunction; Systolic dysfunction; Morphological changes; Apoptosis

Core tip: Changes in metabolic signalling pathways *via* several mediators contribute to the pathogenesis of cardiac dysfunction in diabetes called diabetic cardiomyopathy (DC). In this review, the pathogenetic and pathomorphological changes leading to diastolic and systolic dysfunction in diabetes are discussed. Echocardiographic studies report on the association between diabetes and the presence of cardiac hypertrophy and myocardial stiffness that lead to diastolic dysfunction. More recently reported echocardiographic studies with more sensitive techniques, such as strain analysis, also observed systolic dysfunction as an early marker of DC.

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INTRODUCTION

Since 1972, when Rubler *et al*^[1] described 4 diabetic patients with congestive heart failure and normal coronary arteries, our knowledge of the observed pathomorphological changes of the heart called diabetic cardiomyopathy (DC) has gradually increased^[2,3]. However, the pathohistological changes in DC are not specific^[1-3]. DC has been defined as ventricular dysfunction that occurs independently of hypertension and coronary artery disease (CAD)^[2]. The prevalence of DC is estimated to 60% in well-controlled type 2 diabetic patients^[4,5]. The most useful method for the detection of DC is echocardiography that usually describes cardiac hypertrophy and diastolic dysfunction^[4,5]. DC is a poorly understood entity, however, some mediators leading to abnormalities in myocardial structure, ventricular dysfunction and heart failure have been reported so far^[6]. Patients with diabetes mellitus (DM) are at high risk for developing heart failure^[6]. The spectrum of heart failure syndrome in DC is also not precisely defined despite the usually used definition

of DC as a diastolic heart failure with normal ejection fraction. Anyhow, in different patients several different associated risk factors are observed, such as hypertension and adiposity or associated clinical entities, such as CAD, small vessel disease, autonomic dysfunction and arrhythmias. All of these entities have a significant influence on myocardial structure and function. In this review, the pathogenesis, as well as the prevalence and potential forms of DC and the question whether DC is either a unique specific cardiomyopathy starting with diastolic dysfunction that eventually leads to ventricular dysfunction and heart failure, are discussed.

PREVALENCE

The prevalence of DM is increasing worldwide due to the increase in population, urbanisation, the prevalence of obesity and physical inactivity. The Framingham Heart study showed that DM increased the risk for heart failure 2.4-fold in diabetic men and fivefold in diabetic women compared with age- and sex-matched control subjects^[6]. This risk was independent of hypertension, obesity and CAD. Diabetic patients also have an increased risk for heart failure after myocardial infarction, compared to non-diabetics^[7,8]. However, not only patients with DM, but also patients with higher baseline glucose without diabetes have a higher incidence of heart failure^[6].

Mainly echocardiographic population-based studies report on the association between DM and the presence of cardiac hypertrophy and myocardial stiffness, independently of hypertension^[4,5]. There are basically two pathophysiological processes leading to heart failure in diabetic patients, the first being CAD and the second DC. CAD is increased in patients with DM due to accelerated atherosclerosis associated with risk factors, such as visceral obesity, hypertension, dyslipidaemia, and prothrombotic factors^[7,9]. Despite the increased burden of CAD in diabetic patients, the real prevalence of CAD in DM patients is unknown^[7,9]. Population-based studies reported on the adverse effect of DM on life expectancy, mainly due to cardiovascular disease and also in patients with heart failure^[10].

PATHOGENESIS

Various animal models of DC have proposed several mediators and effectors that are the consequence of altered metabolic signalling pathways and contribute to the pathogenesis of cardiac dysfunction in diabetes.

Hyperglycemia, advanced glycation end products and insulin resistance

DM is characterized by hyperglycemia, hyperinsulinemia, and insulin resistance^[11]. The reduced glucose uptake in the diabetic heart as a result of insulin resistance facilitates a substrate shift towards increased fatty acids oxidation, resulting in reduced cardiac efficiency^[12]. Epicardial adipose tissue (EAT) that covers 80% of the heart

surface and constitutes approximately 20% of the total heart weight have endocrine and paracrine properties that probably interfere with cardiac function. It is speculated that EAT facilitates the development of insulin resistance and cardiac dysfunction^[13]. Glucotoxicity has been proposed in animal models as an important element of myocardial dysfunction. Glucose and collagen interact, and form Schiff bases. The fibrous network is reorganised with the so-called Amadori products. A further chemical modification of Amadori products leads to the formation of macromolecules that are labelled as advanced glycation end products (AGEs). AGEs are a stable form of cross-linked collagen that accumulate in vessel walls and in myocardial tissue and increase diastolic stiffness of the heart and contribute to endothelial dysfunction^[14]. Higher diastolic left ventricular (LV) stiffness was related to both AGE deposition and interstitial fibrosis^[15]. It was observed that serum levels of AGEs correlate with the prolongation of isovolumic relaxation time in patients with diabetes.

Altered substrate metabolism

Metabolic dysregulation in DM also involves fatty acid metabolism. Despite contradictory reports on the level of circulating free fatty acids (FFA), which are elevated in some studies and not in others^[16,17], there is a dys-regulated lipid signalling that leads to an increased FFA metabolism and accumulation of FFA^[18,19]. In parallel, there is a decrease of insulin-mediated glucose uptake. FFA also induced the inhibition of glucose oxidation and resulted in abnormally high oxygen requirements during FFA metabolism. The net result of enhanced fatty acid oxidation and decreased glucose and pyruvate utilization led to the excess of glycolytic intermediates and increased the synthesis of ceramide leading to apoptosis. This process, called gluco-lipotoxicity induced mitochondrial uncoupling, decreased adenosine triphosphate synthesis and mitochondrial dysfunction^[20,21]. Changes in substrate dependence lead to impaired systolic and diastolic function due to the perturbation of myocardial bioenergetics and contraction/relaxation coupling^[21,22].

Increased oxidative stress

Many studies report oxidative stress as a major common factor in the development of DC, however, the exact mechanisms involved in exacerbated reactive oxygen species (ROS) production are not well understood. Studies proposed insulin resistance and increased mitochondrial fatty acid flux that predisposes cardiac mitochondria to ROS overproduction^[23]. In addition to the more important and larger fraction of total cellular ROS that are generated in mitochondria, enzymatic system in cytosol, such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is also modulated by diabetes^[24]. Increased oxidative stress causes cardiomyocyte cell damage, resulting in programmed cell death-apoptosis and fibrosis^[25].

Impaired calcium homeostasis and dysfunction of mitochondria and endoplasmic reticulum

Oxidative stress exacerbates mitochondrial and endoplasmic reticulum (ER) dysfunction and produces subcellular remodelling and abnormalities of calcium handling^[26]. There is calcium imbalance within the diabetic cardiomyocytes, which is characterized by calcium cytosolic overloading and reduced mitochondrial ATP production. The ER, through negative regulation of insulin's metabolic signalling, additionally impairs calcium homeostasis. There is a release of calcium from the ER into cytosol and reduced activity of the sarcoplasmic reticulum calcium pump^[27]. The consequences of these changes are alterations in the calcium sensitivity of regulatory proteins involved in the regulation of the cardiac actomyosin system, leading to impaired left ventricular function^[28]. As an initial dysfunction, researchers observed prolonged diastolic relaxation time, however later on cardiomyocyte apoptosis due to the formation of mitochondrial permeability transition pore has been observed^[29].

Activation of the renin-angiotensin-aldosterone and sympathetic system

Hyperinsulinemia causes overactivation of the renin-angiotensin-aldosterone system^[30]. This leads to cardiac insulin resistance and the activation of mitogen activated protein kinases, which promote fibroblast proliferation while inducing cardiomyocyte fibrosis and apoptosis^[31]. The serum level of aldosterone is increased in the pre-diabetic and diabetic condition and triggers LV hypertrophy, fibrosis and cardiac remodelling^[32]. Both angiotensin II and aldosterone cause increased production of ROS and the activation of NADPH oxidase, and they therefore increase cytosolic oxidative stress^[33]. Aldosterone also aggravates cardiac fibrosis by triggering pro-inflammatory factors through activation of matrix metalloproteinases and the transforming growth factor β (TGF- β)^[34]. There are reports of overactivation of the sympathetic system in the pre-diabetic and diabetic condition that further contributes to metabolic abnormalities. Straznicki observed the association of blunted sympathetic responsiveness and insulin resistance, and disturbed sympathetic neurobiology is characterized by augmented resting sympathetic nervous activity and blunted sympathetic responsiveness to oral glucose ingestion^[35].

STRUCTURAL CHANGES

Anatomic changes observed in DC are characterised by myocyte hypertrophy and myocardial fibrosis^[2,3]. Beside pathohistomorphological findings, left ventricular hypertrophy, defined as an increase in the left ventricular mass by echocardiography or by magnetic resonance imaging has been reported in DC^[2,3].

Fibrosis, necrosis and apoptosis

In DC, fibrosis is attributed to replacement fibrosis caused by myocyte necrosis and to increased interstitial fibrosis. Interstitial fibrosis in DC is driven mainly by

increased accumulation of collagen type III^[3,36]. DC is characterised by accelerated myocyte cell death and accelerated apoptosis^[3,36]. The processes of accelerated necrosis and apoptosis are driven by hyperglycemia, accelerated production of ROS, upregulation of the local renin angiotensin aldosterone system, and through modulation of the insulin-like growth factor-1 and the TGF- β by angiotensin II. Apoptosis does not cause scar formation or accumulation of interstitial collagen, with nuclear fragmentation and cell shrinkage being replaced by the surrounding cells^[37]. On the contrary, myocyte necrosis produces the widening of extracellular compartments among myocytes and increased deposition of collagen, resulting in replacement fibrosis and connective cell proliferation^[38]. The presence of hypertension in patients with diabetes increases myocyte necrosis 1.4-fold compared to diabetes alone, but it has no influence on apoptosis^[39].

Cardiomyocyte hypertrophy

In DC, Huyn and Rosenkrans observed the increase of several markers of cardiomyocyte hypertrophy, including increased cardiomyocyte width and myofiber disarray^[40,41]. The loss of cardiomyocytes due to apoptosis and necrosis lead to compensatory hypertrophy of the remaining viable cardiomyocyte. Researchers observed an upregulation of hypertrophic gene expression of β -myosin heavy chain, ANP, and BNP. The causes of the diabetes-induced hypertrophic response are probably hyperglycemia and oxidative stress^[40,41].

CHANGES IN CARDIAC FUNCTION

Diastolic dysfunction

A number of echocardiographic studies have characterised functional changes early in the course of DC. Diastolic abnormalities have been reported in 23% to 75% of patients with DM^[42-45]. A high variability in the prevalence of diastolic dysfunction raises a question on the implemented methodology. Most of the patients included in these studies were asymptomatic without overt heart disease and their report based on mitral inflow pattern where they observed an increased E/A ratio (where E is mitral peak early-diastolic filling velocity; A is mitral late diastolic filling velocity), prolonged deceleration time, increased isovolumic relaxation time, or described combined indices derived from mitral inflow pattern and pulmonary venous flow^[46-48]. Later on, some investigators analysed Doppler tissue imaging diastolic velocities and mitral inflow pattern and reported on their indices, such as E/e' that is non-invasive correlate of left ventricular filling pressure (e' is the early diastolic mitral annular velocity)^[45,49]. Ernande reported a 47% prevalence of diastolic dysfunction, with 33% grade I or pattern of impaired relaxation and 14% grade II or pseudonormal pattern^[50] in patients with DM with normal ejection fraction and controlled blood pressure. Anyhow, most of these studies have been completed before the reliable complex diagnostic algorithm of diastolic function was accepted, and therefore did not allow us a conclusion based on

single parameters^[51].

Systolic dysfunction

Although many studies have shown that diabetic patients have abnormal diastolic function but preserved systolic function, this may well be due to techniques used for the evaluation of systolic and diastolic dysfunction. Usually applied techniques are probably more sensitive for diastolic dysfunction than for systolic dysfunction. When thinking of systolic function, we usually think of ejection fraction that depends a lot on radial contractile function, but the longitudinal contractile function of ventricle is primary depressed. Moreover, with the application of more sensitive techniques for the analysis of systolic function, such as strain deformation imaging, researchers observed that the systolic function is impaired despite normal left ventricular ejection fraction. Ernande reported that preclinical radial and longitudinal systolic strain is depressed in 28% of patients with DM with normal diastolic function^[50]. This study indicates that systolic strain alteration may exist despite normal diastolic function, or otherwise indicating that diastolic dysfunction should not be considered the first marker of a preclinical form of DC.

Continuum of diastolic and systolic dysfunction

Deterioration of systolic and diastolic function is continuum. There is no separation of diastolic and systolic function in DM, nor in other metabolic cardiomyopathies. Diastolic dysfunction was associated with increased cardiac triglyceride content in the ob/ob mice model of DM^[52]. The role of calcium homeostasis studied in the db/db mice model of DM showed increased diastolic sarcoplasmic reticulum Ca²⁺ leak, reduced synchrony of Ca²⁺ release, lower peak systolic and diastolic Ca²⁺ have, therefore, an influence on both systolic and diastolic function^[53]. Abnormality in systolic and diastolic function is also associated with myocardial structural changes. Obviously, there are numerous factors that might have an unfavourable effect on systolic and diastolic function in subjects with DM.

PHENOTYPE OF DC

There is still a debate on how DC should be defined. DC is not an isolated diastolic entity. Due to metabolic abnormalities, we observed systolic and diastolic dysfunctions that are initially subclinical and gradually progress to a full-blown syndrome of congestive heart failure. To conclude, according to current knowledge, DC is expected to be a common single phenotype that is caused by different pathogenetic and pathomorphological changes leading to diastolic and systolic dysfunction in diabetes.

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Primary reperfusion in acute right ventricular infarction: An observational study

Eulo Lupi-Herrera, Héctor González-Pacheco, Úrsulo Juárez-Herrera, Nilda Espinola-Zavaleta, Eduardo Chuquiure-Valenzuela, Ramón Villavicencio-Fernández, Marco Antonio Peña-Duque, Ernesto Ban-Hayashi, Sergio Férrez-Santander

Eulo Lupi-Herrera, The American British Cowdray Medical Center I.A.P., Mexico City 14080, Mexico

Sergio Férrez-Santander, Subdivisions of Clinical-Research and Teaching, National Institute of Cardiology Ignacio Chavez, Mexico City 14080, Mexico

Héctor González-Pacheco, Úrsulo Juárez-Herrera, Eduardo Chuquiure-Valenzuela, Coronary Care Unit, National Institute of Cardiology Ignacio Chavez, Mexico City 14080, Mexico

Nilda Espinola-Zavaleta, Ramón Villavicencio-Fernández, Marco Antonio Peña-Duque, Ernesto Ban-Hayashi, Echocardiography and Interventional Cardiology Departments, National Institute of Cardiology Ignacio Chavez, Mexico City 14080, Mexico

Author contributions: Lupi-Herrera E designed, performed the research, analyzed the obtained data and wrote the manuscript; González-Pacheco H, Juárez-Herrera Ú, Chuquiure-Valenzuela E performed the research, wrote the manuscript and analyzed the obtained data; Villavicencio-Fernández R, Peña-Duque MA, and Ban-Hayashi E performed the cardiac catheterization studies and primary percutaneous coronary intervention; Espinola-Zavaleta N analyzed the echocardiographic studies, provided new reagents and also were involved in writing and editing the manuscript; Férrez-Santander S was involved in editing the manuscript.

Correspondence to: Eulo Lupi-Herrera, MD, FACC, Director of the Cardiovascular Division, The American British Cowdray Medical Center I.A.P., Sur 136-116, Col Las Américas, Mexico City 14080, Mexico. elupih@abchospital.com

Telephone: +52-33-308000 Fax: +52-33-308000

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classified as without right ventricular failure (RVF) (class A, $n = 425$, 64%), with RVF (class B, $n = 158$, 24%) or with cardiogenic shock (CS) (class C, $n = 96$, 12%). Of the 679 patients, 148 (21.7%) were considered to be eligible for thrombolytic therapy (TT) and 351 (51.6%) for primary percutaneous coronary intervention (PPCI). TIMI 3-flow by TT was achieved for A, B and C RVI class in 65%, 64% and 0%, respectively and with PPCI in 93%, 91% and 87%, respectively.

RESULTS: For class A without RT, the mortality rate was 7.9%, with TT was reduced to 4.4% ($P < 0.01$) and with PPCI to 3.2% ($P < 0.01$). Considering TT vs PPCI, PPCI was superior ($P < 0.05$). For class B without RT the mortality was 27%, decreased to 13% with TT ($P < 0.01$) and to 8.3% with PPCI ($P < 0.01$). In a TT and PPCI comparison, PPCI was superior ($P < 0.01$). For class C without RT the in-hospital mortality was 80%, with TT was 100% and with PPCI, the rate decreased to 44% ($P < 0.01$). At 8 years, the mortality rate without RT for class A was 32%, for class B was 48% and for class C was 85%. When PPCI was successful, the long-term mortality was lower than previously reported for the 3 RVI classes (A: 21%, B: 38%, C: 70%; $P < 0.001$).

CONCLUSION: PPCI is superior to TT and reduces short/long-term mortality for all RVI categories. RVI CS patients should be encouraged to undergo PPCI at a specialized center.

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Key words: Right ventricular infarction; Reperfusion therapy; Ventricular failure; Cardiogenic shock; Morbidity; Mortality

Core tip: It is, up to our knowledge the largest series of

Abstract

AIM: To investigate the impact of primary reperfusion therapy (RT) on early and late mortality in acute right ventricular infarction (RVI).

METHODS: RVI patients ($n = 679$) were prospectively

acute right ventricular infarction (RVI) patients where all the clinical RVI spectrum is considered. RVI is analyzed in relation to primary reperfusion procedures, over a study period with a more widespread use of primary percutaneous coronary intervention (PPCI) together with the advent of stents and antiplatelet agents to provide a better insight into reperfusion trends and results in acute RVI. According to our findings, in all RVI hemodynamic scenario PPCI is superior to thrombolytic therapy (TT) and reduces short and long-term mortality for all 3 RVI categories. Patients in cardiogenic shock should be encouraged to undergo PPCI rather than TT at a specialized center.

Lupi-Herrera E, González-Pacheco H, Juárez-Herrera Ú, Espino-la-Zavaleta N, Chuquique-Valenzuela E, Villavicencio-Fernández R, Peña-Duque MA, Ban-Hayashi E, Férrez-Santander S. Primary reperfusion in acute right ventricular infarction: An observational study. *World J Cardiol* 2014; 6(1): 14-22 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i1/14.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i1.14>

INTRODUCTION

Right ventricular infarction (RVI) is relatively common in patients with acute inferior-posterior left ventricular myocardial infarction (IPLVMI). RVI can depress right ventricular (RV) function, resulting in right ventricular failure (RVF) or cardiogenic shock (CS)^[1-5]. There are scarce and somewhat conflicting clinical data concerning the effects of interventions designed to achieve reperfusion of the RV myocardium in acute ischemia. Several investigators have suggested that RV function improves only after successful thrombolytic therapy (TT), whereas others have reported recovery in the absence of early or even any reperfusion of the right coronary artery (RCA)^[6]. In a study involving limited number of patients, rapid hemodynamic improvement and excellent clinical outcomes have been reported after successful primary percutaneous coronary intervention (PPCI) of the RCA and its major RV branches^[1]. At the most extreme end of the hemodynamic spectrum for RVI, when CS is analyzed in relation to reperfusion, the results can be disappointing, partly due to the time frame of the study (1993-1998), or the results can be better than the outcomes in patients with left ventricular (LV) pump failure in a study from 1984 to 2004^[7]. The present study aimed (1) to evaluate the trends and impact of TT and PPCI over time and on early and late mortality in RVI patients with or without RVF; and (2) with CS over a study period with a more widespread use of PPCI together with the advent of stents and potent antiplatelet agents to provide a better insight into reperfusion results in acute RVI.

MATERIALS AND METHODS

Ethics

This work has been carried out in accordance with the

Declaration of Helsinki (2000) of the World Medical Association. The protocol was approved by the ethics committee of the institution. All patients provided informed written consent.

Patients

We prospectively screened 2679 consecutive patients admitted with a first acute (defined as the time from symptom onset to admission of ≤ 48 h) IPLVMI (defined as chest pain with > 1 mm in leads II, III and aVF) from January 1996 to March 2009, and identified 679 (25.3%) patients with infarction extending to the walls of the right ventricle (84% were studied in the last 10 years). Isolated acute RVI, history of valve heart disease, and previous heart or renal failure were exclusion criteria for this study.

The diagnostic criteria for IPLVMI with extension to the walls of the right ventricle, RVF and CS have been published previously^[4]. Briefly, in addition to standard electrocardiographic (EKG) leads, a right-sided precordial EKG (leads V_{3R}-V_{7R}) was recorded in all patients immediately after admission. An ST-segment elevation in lead V_{3R} or V_{4R} of greater than 0.1 mV was used to diagnose RVI. The diagnosis of RVI was also based on clinical features that have been described and have been associated with this variety of infarction, and on echocardiographic findings^[2]. The diagnosis of RVF was based on clinical features, including persistent systemic hypotension [systemic systolic pressure (SSP) ≤ 100 mmHg, right-sided S₃ and S₄] without features of shock, echocardiographic evidence of ischemic RVF (RV wall motion abnormalities (WMA) associated with gross RV dilatation), findings that suggest globally depressed RV function and invasive hemodynamic monitoring identifying RVI by a combination of findings that suggest RV dysfunction [low cardiac output (CO) and a disproportionate elevation of the mean right atrial pressure (mRAP) compared to the mean pulmonary wedge pressure (mPWP)]^[2,8,9]. The diagnosis for shock was made if all of the following criteria were satisfied: SSP persistently ≤ 90 mmHg or vasopressors required to maintain SSP > 90 mmHg; very low CO [cardiac index (CI) < 2.1 L.min/m²]; evidence of end-organ hypoperfusion. We did not include in this category patients with hypotension related to hypovolemia, transient hypotension due to vasodilatation and bradycardia associated with spontaneous reperfusion (Bezold-Jarisch reflex), or hypotension due to atrioventricular block (AVB), cardiac arrhythmias or mechanical complications (ventricular septal or myocardial wall rupture or cardiac tamponade) at admission.

We risk-stratified RVI patients into 3 subsets based on clinical features, echocardiographic findings upon admission and hemodynamic findings as follows: class A (without RVF), comprised of patients without evidence of systemic hypotension (SSP ≥ 100 mmHg) or RVF (by clinical features, echocardiographic and/or hemodynamic findings); class B (with RVF), those with persistent systemic hypotension (SSP < 100 mmHg) or RVF, but without other clinical features of shock; class C, those with

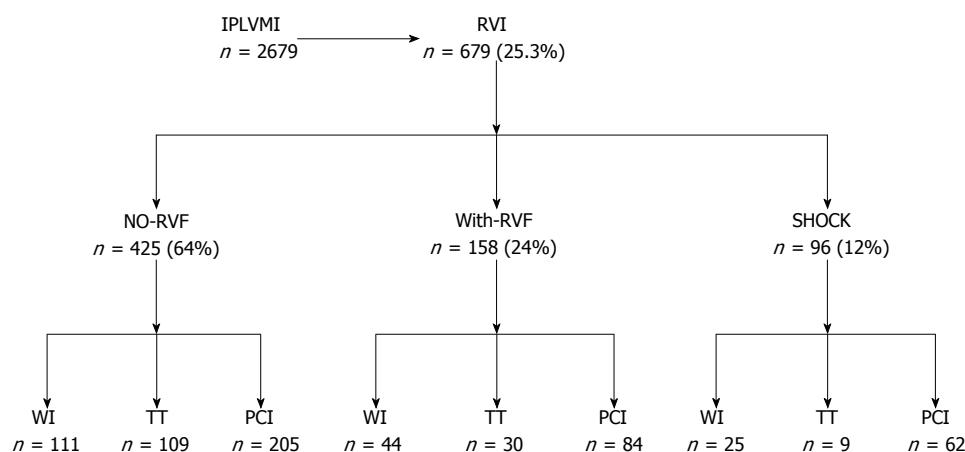


Figure 1 Study outline. Patients with inferior-posterior left ventricular myocardial infarction (IPLVMI) ($n = 2679$), with right ventricular infarction (RVI): 679 (25.3%). We risk-stratified RVI patients into 3 subsets: class A (without RVF), class B (with RVF) and class C (with cardiogenic shock). WI: Without intervention; TT: Thrombolytic therapy; PCI: Primary coronary intervention; RVF: Right ventricular failure.

CS (Figure 1).

Evaluation of atrial and ventricular function

Echocardiograms were analyzed according to previous methods^[4]. LV systolic dysfunction was defined as an ejection fraction below 50%. The RV was divided into eight segments; of these segments 3 corresponded to the basal segments, 3 to the middle segments of the anterior, lateral and inferior walls (IW), and 2 to the apical anterior and inferior segments. The wall movement index (WMI) of both ventricles was obtained by assigning a value to the movement of each wall segments and dividing the sum by the number of segments corresponding to the ventricle in question. A score of 0 was classified to normal movement, 1 as = hypokinesis (diminished thickening), 2 as = akinesis (absence of thickening) and 3 as = dyskinesis (paradoxical systolic movement). The overall score for RV and LV wall motion (LVWM) was calculated as the average score for the segments, and the ratio of the diastolic diameters of the two ventricles diastolic ratio of the two ventricles (RVD/LVD) was also calculated. The echocardiographic evidence of ischemic RV was based on a combination of the following features: right ventricular wall motion abnormalities (RVWMA) and RV dilatation with/without tricuspid regurgitation (TR). Right atrial (RA) ischemia was defined according to the following criteria: akinesis of the RA free wall (FW) despite left atrial contraction, thrombosis at the site of akinesis, presence of spontaneous contrast in the RA, and inversion of the interatrial septal convexity^[4].

Evaluation of coronary anatomy and perfusion

The location of the culprit lesion was assessed using Bowers criteria^[1]. The initial and post-reperfusion flow grades in the coronary artery and in the RV branches were scored from 0 to 3 [thrombolysis in myocardial infarction (TIMI) flow classification]. Successful reperfusion was defined as < 50% residual stenosis and restoration of TIMI grade 3 in the main RCA and its major RV branches (> 1 mm). Coronary collateral blood flow was

evaluated according to Rentrop *et al.*^[10]. Multivessel disease was defined as a lesion $\geq 50\%$ in ≥ 2 major coronary arteries. We analyzed all of the patients who had a coronary angiogram at admission and a second angiogram after reperfusion. For those who received TT, the second coronary angiogram was performed 90-180 min after the thrombolytic infusion. Information on the coronary arteries was evaluated independent of and blinded to the other study data.

Treatment protocol

Patients were treated with unfractionated heparin (bolus: 60 IU/kg, maximum 5000 IU; 10 IU/kg per hour, maximum 1000/h) and aspirin (300 mg) in the emergency room and transferred to the coronary care unit or to the catheterization laboratory to undergo coronary angiography, based on the individual's. Patients who were eligible for reperfusion were consecutively assigned to TT or PPCI. TT was administered intravenously to all of the eligible RVI patients. The inclusion criteria for classes A and B were defined before admission as follows: symptoms of ST elevation myocardial infarction (STEMI) lasting less than 12 h or > 12 h in patients with ongoing ischemia, age ≤ 75 years and absence of other accepted contraindications. TT was performed with either streptokinase (32%) or recombinant tissue plasminogen activator (68%), preceded by heparin. Post-TT consisted of an intravenous heparin dose that was adjusted to maintain TPTa between 60-75 s for 72 h and aspirin. For PPCI, the same inclusion criteria for STEMI symptoms and duration and age were applied; stents were deployed according to standard techniques followed by standard antiplatelet therapy. Stents and glycoprotein II b/IIIa platelet inhibitors became a standard therapy in eligible patients in 1995 and 1996, respectively. Clopidogrel was used in stented patients with a loading dose of 300 mg or 600 mg and later, a daily dose of 75 mg for ≥ 6 mo in patients treated with bare metal stents and for 6 to 12 mo in patients treated with drug-eluting stents. RVI patients in shock were considered to be candidates for PPCI if age

Table 1 Clinical characteristics of the 679 patients separated according to right ventricular infarction class

Variables	Class A <i>n</i> = 425 (64%)	Class B <i>n</i> = 158 (24%)	Class C <i>n</i> = 96 (12%)
Age (yr)	59.7 ± 9.4	61 ± 10	62.4 ± 8.4
Age 64-74 yr	41.9%	38.7%	37.5%
Men	82%	81%	83%
Pre-IA	38%	40%	41%
Peak CK IU/L	1854	1994	2109
median (25 th -75 th percentiles)	(1654-2490)	(1214-2634)	(1532-2812)
Peak MB fraction IU/L	188	201	192
median (25 th -75 th percentiles)	(111-350)	(126-312)	(104-333)
Diabetes	26.9%	27.8%	30.6%
Hypertension	40.7%	44.3%	44.4%
Hypercholesterolemia	23%	19%	21%
Smoking	58%	60%	55%
BMI (kg/m ²)	28.2 ± 4.4	26.7 ± 5.2	27.2 ± 3.9

No statistically significant differences between right ventricular infarction classes were found. Pre-IA: Infarction angina; CK: Creatine kinase; BMI: Body mass index; MB: Myocardial B.

< 75 years and shock developed within ≤ 48 h of the STEMI.

Conservative therapy for A and B class was provided to patients who delayed seeking medical attention, for class C > 75 years of age or those who developed shock > 48 h after the onset of myocardial infarction. Patients underwent PPCI or coronary artery bypass graft surgery (CABGS) only if ischemia recurred despite medical therapy during hospitalization or occurred during a pre-discharge stress test.

Morbidity and mortality analysis

The following adverse cardiac events were recorded: hypotension (> 1 h), hypotension necessitating volume infusion (200-400 mL/h), pharmacological hemodynamic support (inotropic agents/vasopressors), or intra-aortic balloon pump (IABP); high grade AVB (> 1 h, need for a transient pacemaker); ventricular arrhythmias requiring treatment; recurrent ischemia (defined as recurrent chest pain with or without new EKG changes or recurrent myonecrosis as indicated by serum biomarkers (increase in creatine kinase level and MB fraction higher than the nadir); death. Urgent target vessel revascularization was defined as the need to repeat PPCI or urgent CABGS for recurrent ischemia or hemodynamic compromise during the hospital stay.

The primary end point was in-hospital cardiac death at 30 d. Follow-up information after hospital discharge was obtained from the hospital records database, which is updated at each patient visit and upon patient death.

Statistical analysis

We analyzed: (1) the clinical, echocardiographic, angiographic and hemodynamic characteristics of our patients at baseline separately for those in classes A, B, and C; (2) among the 3 RVI classes; (3) within each class, all patients sent for reperfusion were compared to those who were

not referred for reperfusion; and (4) within each class, TT vs PPCI was compared.

The continuous data are expressed as the mean ± SD unless otherwise specified. A Student's *t* test, 1-way-ANOVA (Bonferroni's-test for multiple comparisons), χ^2 , or Fisher exact test was used as appropriate.

Univariate analysis based on the logistic regression model was used to examine the relationship between the selected demographic, medical history, clinical examination, and hemodynamic data to determine the likelihood of overall mortality. After the univariate analysis, any variable that had a univariate test value of $P < 0.25$ was considered to be a candidate for the multivariate analyses. The results are expressed as odds ratios and 95%CI. The Kaplan-Meier method was used to estimate the overall survival distribution (log-rank test). The analyses were performed using the STATA-9 software.

RESULTS

Clinical data

From all of the RVI patients, echocardiography, invasive hemodynamic evaluation and coronary angiograms were performed in 94.5%, 89% and 73%, respectively. The diagnosis of RVI was made by EKG, echocardiographic, hemodynamic or coronary angiographic criteria in 100% and by 3 criteria (echocardiographic, hemodynamic and angiographic) in 85% of the patients. The RVI subgroups had no differences in their baseline clinical characteristics (Table 1).

Echocardiography

At baseline, there was evidence of IPLVMI dysfunction in all of the RVI patients (WMI 1.8 ± 0.4). The echocardiographic data at baseline by clinical class was documented as follows. In all of the classes A patients without RV-dilatation, WMA were only found in the IW (LV + RV) = 84% and the FW = 16%. In class B and C patients with RV-dilatation, WMA was not only confined to the IW; TR, and abnormal ventricular septal movement were observed in 78% and 70%, respectively, with abnormal RA wall movement in 18% and 25%, respectively. Abnormal values for the right ventricular motion index were found in all of the patients, with a significant increase in the WMA score classes between A, B and C classes ($P < 0.01$, respectively). An RVD/LVD of = 1 was most frequently observed in class B, and an RVD/LVD > 1 was observed in C patients (Table 2).

Hemodynamics

The hemodynamic data according to the RVI class ($n = 604$, 89%) was documented as follows. In class A patients ($n = 350$), all of the hemodynamic parameters were within normal limits. In class B patients ($n = 158$), an elevated mRAP (12.9 ± 3.6 mmHg) and decreased CI (2.4 ± 0.21 L.min/m²) and mean systemic arterial pressure [mSAP (78.7 ± 12.3 mmHg)] were found, with an increased RAP/PWP > 0.8 compared to class A patients.

For class C patients ($n = 96$), an elevated mRAP (21.4

Table 2 Echocardiographic data at baseline according to right ventricular infarction class

Variable	Class A (n = 396)	Class B (n = 152)	Class C (n = 94)	Total (n = 642)
RV-dilatation	0%	100% ^b	100%	38.3%
VWMA	100%	100%	100%	100%
WMA only for IW	84%	0%	0%	51.7%
WMA for IW + OW	16%	100% ^b	100%	48%
TR	17%	100% ^b	100%	48%
AVSM	25%	78% ^b	70%	43.9%
RVTI	1.9% ± 0.3%	2.5% ± 0.2% ^b	3.4% ± 0.5% ^d	2.4% ± 4%
RVD/LVD > 1	0%	25% ^b	83% ^d	18%
RVD/LVD = 1	0%	65% ^b	17% ^d	18%
ARAWM	0%	18% ^b	25%	8%
RA-DPS n (%)	0 (0)	19 (70)	10 (43) ^d	29 (58)
LVEF < 0.5	11%	22%	34% ^d	16%

Echocardiographic data at baseline according to right ventricular infarction class (n = 642/679, 94.5%); transthoracic (TT): 375 (58%), transesophageal (TE): 267 (42%). ^bP < 0.01 vs classes A; ^dP < 0.01 vs classes B. RV: Right ventricle; VWMA: Ventricular wall motion abnormalities; IW: Inferior wall for LV + RV; OW: Other walls; TR: Tricuspid regurgitation; AVSM: Abnormal ventricular septal motion; RVTI: Right ventricular motion index (mean ± SD); RVD/LVD: Diastolic ratio of the two ventricles; ARAWM: Abnormal right atrial wall movements; RA-DPS: Right atrium dobutamine positive stress; LVEF: Left ventricular ejection fraction.

Table 3 Hemodynamic data at baseline according to the right ventricular infarction class

Variable	Class A (n = 350)	Class B (n = 158)	Class C (n = 96)
mRAP (mmHg)	4.6 ± 2.1	12.9 ± 3.6 ^b	21.4 ± 5.15 ^d
REVD (mmHg)	3.4 ± 1.7	11.2 ± 4 ^b	16 ± 5.4 ^d
sPAP (mmHg)	16.2 ± 4.4	16.4 ± 3.9	36.8 ± 9.3 ^d
dPAP (mmHg)	9.7 ± 3.7	13.4 ± 2.2	22.2 ± 3.9 ^d
mPWP (mmHg)	8.6 ± 3.1	12.1 ± 1.8	19.9 ± 6.2 ^d
CI (L.min/m ²)	3.4 ± 0.71	2.4 ± 0.21 ^b	1.67 ± 0.5 ^d
mSAP (mmHg)	108.8 ± 7	78.7 ± 12.3 ^b	62.7 ± 9.5 ^d
RAP/PWP ≥ 0.8	2%	96% ^b	92%

Hemodynamic data at baseline according to the right ventricular infarction class (n = 604, 89%). ^bP < 0.01 vs classes A; ^dP < 0.01 vs classes B. m: Mean; s: Systolic; d: Diastolic; RAP: Right atrial pressure; REVD: Right ventricular end diastolic pressure; PAP: Pulmonary artery pressure; PWP: Pulmonary wedge pressure; CI: Cardiac index; SAP: Systemic arterial pressure.

± 5.15 mmHg), systolic pulmonary artery pressure [sPAP (36.8 ± 9.3 mmHg)], diastolic PAP (22.2 ± 3.9 mmHg) and mPWP (19.9 ± 9.2 mmHg) and decreased CI (1.67 ± 0.5 L.min/m²) and mSAP (62.7 ± 9.5 mmHg) were found compared to class B patients (Table 3).

Angiography

The RCA was the infarct-related artery in 95% of the RVI patients. There was severe compromise of RV perfusion, as indicated by the TIMI grade flow (0.7 ± 1).

The culprit RCA lesion was most commonly found proximal in class B and C patient and had a mid location in class A patients. The 3 RVI subgroups had no differences in prevalence of 1 and 2 vessels disease (VD), but 3-VD was more frequently observed in classes B and C.

Table 4 Coronary angiographic data at baseline according to the right ventricular infarction class n (%)

Variable	Class A (n = 425)	Class B (n = 158)	Class C (n = 96)	Total
Angiography	291 (68)	123 (77)	85 (88)	499 (73)
PCI	213	93	76	382 (76.5)
TT	78	30	9	117 (23.4)
RCA culprit vessel	266 (91)	123 (100)	85 (100)	474 (95)
RCA CL-location				
Proximal	42 (16)	85 (69) ^b	78 (92) ^d	205 (43)
Mid	144 (54)	26 (21) ^b	6 (7) ^d	176 (37)
Distal	80 (30)	12 (9) ^b	1 (1) ^d	93 (19)
1-VD	144 (54)	39 (32) ^b	11 (14)	194 (40.9)
2-VD	96 (36)	31 (25)	32 (37)	159 (33.5)
3-VD	26 (9)	53 (43) ^b	42 (49)	121 (25.5)
RCA + LAD-D	29 (11)	44 (36) ^b	45 (53) ^d	118 (24.8)
100% RCA-O	119 (45)	68 (55)	53 (62)	240 (48)
Without CCC	47 (40)	36 (53)	47 (89) ^d	130 (54)
With CCC	72 (60)	32 (47)	6 (11) ^d	110 (45)
Grade 1	9	10	0	19
Grade 2	36	12	0	48
Grade 3	27	10	6	43
RCA-RVB-RAB flow				
TIMI grade flow	0.8 ± 1.4	0.7 ± 1	0.6 ± 0.7	0.7 ± 1
TIMI 3 flow in all RVB	194 (72)	0 (0) ^b	0 (0)	194 (40)
Impaired flow in 1 RVB	56 (21)	11 (8.9) ^b	0 (0)	67 (14)
Impaired flow in ≥ 2				
RVB	16 (6)	41 (33) ^b	6 (7) ^d	63 (13)
No flow in all RVB	0 (0)	71 (57) ^b	79 (92) ^d	150 (31)
RAB with no flow	0 (0)	19 (15) ^b	29 (34) ^d	48 (10)

Coronary angiographic data at baseline according to the right ventricular infarction class (n = 499/679, 73%). ^bP < 0.01 vs classes A; ^dP < 0.01 vs classes B; TIMI: Thrombolysis in myocardial infarction. V: Vessel; D: Disease; O: Obstruction; RCA: Right coronary artery; CL: Culprit lesion; CCC: Coronary collateral circulation; RVB: Right ventricular branch; RAB: Right atrial branch; PCI: Primary coronary intervention; LAD-D: Left anterior descending diseases; TT: Transthoracic.

The combination of RCA and significant left anterior descending (LAD > 50% stenosis) disease was most commonly found in C patients (53%), and it was significantly different from A (11%) and B (36%) patients (P < 0.01).

Complete RCA obstruction was found in 240 patients (48%), and 45% of these patients had coronary collaterals. Significant differences in collateral blood flows were found among A, B, and C patients (60%, 47% and 11%, respectively, P < 0.01). None of the class A patients demonstrated an absence of flow to all of the RV or RA branches. For class B and C patients, no flow to all of the RV or RA branches was observed in 57% and 15%, and 92% and 34%, respectively (Table 4).

Reperfusion

Of the 679 RVI patients, 148 (21.7%) were eligible for TT and 351 (51.6%) for PPCI. TIMI 3-flow by TT was achieved for 65%, 64%, and 0% for RVI classes A, B, and C. TIMI 3-flow was achieved with PPCI in 93%, 91%, and 87%, respectively. The mean residual coronary artery lesion at 90-180 min for the group RVI patients treated with TT or PPCI was 68% ± 10% and 10% ± 8%, respectively (P < 0.000).

Table 5 Procedural variables and results of primary reperfusion at entry according to right ventricular infarction class (*n* = 679)

Variable	Class A (<i>n</i> = 425)	Class B (<i>n</i> = 158)	Class C (<i>n</i> = 96)	Total
Symptoms to admission time (min-max) h	4.6 (2-23)	5.5 (3.4-19)	27 (19-48) ^d	
Medical treatment	111 (26)	44 (27)	25 (26)	180 (26.5)
TT	109 (25)	30 (19) ^b	9 (9) ^d	148 (21.7)
Primary PCI	205 (48)	84 (53)	62 (64)	351 (51.6)
Stent use	182 (88)	80 (95)	62 (100)	324 (92)
II b/ III a GI use	49%	47%	52%	
Inotropes/vasopressors	0%	100% ^b	100%	
Temporary pacemaker	1.20%	10.7% ^b	29% ^d	
IABP support	0	0	73.9% ^d	
MVA	0	0	100% ^d	
Median time from MI to reperfusion treatment (min-max) h	1.9 (1.3-4)	2.1 (1.4-3.8)	14 (8-22) ^d	
Door-to-needle time (min)	42 ± 18	48 ± 16	-	
Door-to-balloon time (min)	93 ± 24	89 ± 37	198 ± 102 ^d	
TIMI 3 flow after PCI	93%	91%	87%	
TIMI 3 flow after TT	65%	64%	0% ^d	
RCA-Extensive clot burden	17%	22%	75% ^d	
At 24-48 h reversal of RVWMA with SR	84%	78%	69% ^d	

^b*P* < 0.01 *vs* classes A; ^d*P* < 0.01 *vs* classes B. TT: Transthoracic; PCI: Primary coronary intervention; IABP: Intra-aortic balloon pump; MVA: Mechanical ventilatory assistance; RCA: Right coronary artery; SR: Success reperfusion; RVWMA: Right ventricular wall motion abnormalities; GI: Glucoprotein inhibitors; MI: Myocardial infarction; TIMI: Thrombolysis in myocardial infarction.

Table 6 Hospital outcomes based on reperfusion for each RVI class *n* (%)

Variable	Class A				Class B				Class C			
	NR (<i>n</i> = 111)	TT (<i>n</i> = 109)	PCI (<i>n</i> = 205)	Total (<i>n</i> = 425)	NR (<i>n</i> = 44)	TT (<i>n</i> = 30)	PCI (<i>n</i> = 84)	Total (<i>n</i> = 158)	NR (<i>n</i> = 25)	TT (<i>n</i> = 9)	PCI (<i>n</i> = 62)	Total (<i>n</i> = 96)
AVB+	16 (14)	7 (6) ^b	11 (5)	34 (8)	14 (31)	9 (30)	6 (7) ^d	29 (18) ^b	22 (88)	8 (88)	13 (20)	43 (45) ^f
SVT/VF+	18 (16)	6 (5) ^b	9 (4)	33 (8)	18 (40)	9 (30)	4 (4.7) ^d	31 (19.6) ^b	14 (56)	5 (55)	9 (14) ^d	28 (29) ^f
AF/PAT	4 (3)	3 (2.7)	0 (0)	7 (1.6)	5 (11)	6 (20)	4 (4.7) ^d	15 (9.4) ^b	4 (16)	2 (22)	5 (8)	11 (11)
R-MI+	6 (5)	8 (7.3)	2 (0.9)	16 (4)	4 (9)	3 (10)	1 (1)	8 (5)	2 (8)	2 (22)	3 (4.8)	7 (7.2)
UTVR+	6 (5)	4 (3.6)	2 (0.9)	12 (3)	3 (6)	1 (3)	1 (1)	5 (3)	2 (8)	1 (11)	3 (4.8)	6 (6)
MR/T+	0 (0)	2 (1.8)	0 (0)	2 (0.4)	4 (9)	2 (6)	0 (0)	6 (3.7)	4 (16)	2 (22)	2 (3)	8 (8.3)
ARF	4 (3)	3 (2.7)	2 (0.9)	9 (2)	4 (9)	2 (6)	4 (4.7)	10 (6)	5 (20)	3 (33)	10 (16)	18 (19)
SSH+	18 (16)	2 (1) ^b	0 (0)	20 (5)	-	-	-	-	-	-	-	-
E-CS+	8 (7)	6 (5)	0 (0)	14 (3.2)	10 (22)	4 (13) ^b	8 (9)	22 (14) ^b	-	-	-	-
Death	9 (7.9)	5 (4.4) ^a	5 (3.2) ^f	19 (4.4)	12 (27)	4 (13) ^b	7 (8.3) ^d	23 (14.5) ^b	20 (80)	9 (100)	27 (44) ^d	56 (58) ^f

^a*P* < 0.05, ^b*P* < 0.01 *vs* NR; ^d*P* < 0.01 *vs* primary coronary intervention (PCI); ^f*P* < 0.01 *vs* classe B; ^b*P* < 0.01 *vs* classe C. NR: Not send to reperfusion; TT: Transthoracic; AVB: Atrio-ventricular block requiring pacing; SVT: Sustained ventricular tachycardia; VF: Ventricular fibrillation; AF: Atrial fibrillation; PAT: Paroxysmal atrial tachycardia; R-MI: Reinfarction of the myocardium; UTVR: Urgent target vessel revascularization; MR/T: Myocardial rupture/tamponade; ARF: Acute renal failure; SSH: Sustained systemic hypotension (lasting < 12-24 h reversed with volume infusion and inotropic support); E-CS: Evolution to cardiogenic shock; +: Considered to be major cardiac complications.

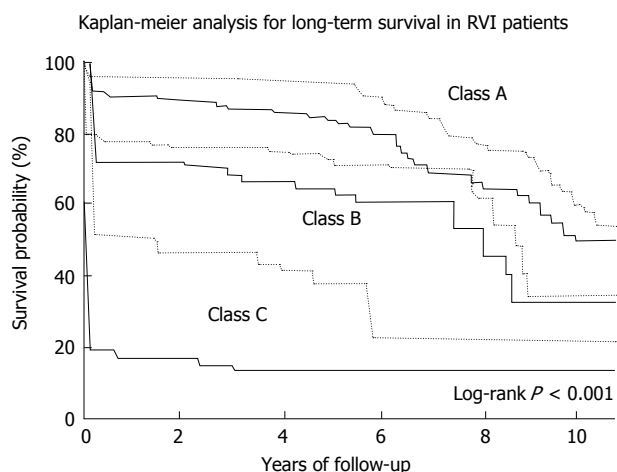
Reversible RVWMA at 24-48 h with successful reperfusion was observed for classes A, B, and C patients in 84%, 78%, and 69%, respectively. Reversible RVF (defined as the normalization of the SSP without volume infusion or inotropic agents and/or improvement or normalization of RVWMA and RV dilatation by echocardiography) was documented for class B in 83% and for CS in 43% (Table 5).

Outcome

Class A: Half of the patients had uneventful clinical courses, although 3.2% developed CS, and 4.4% died. Hemodynamic data recorded at 48-72 h in 134 patients with or without successful reperfusion did not show any significant changes compared to baseline hemodynamics measurements. For those patients who received conser-

vative treatment the mortality rate was 7.9%. With TT or PPCI, decreased rates of bradyarrhythmias, AVB, ventricular arrhythmias and in-hospital mortality were observed. After PPCI, no patient progressed to CS, and significantly fewer patients died compared to those treated with TT (*P* < 0.01 and *P* < 0.05, respectively) (Table 6).

Class B: PPCI was associated with a decrease in AVB and ventricular arrhythmias. When reperfusion was successful, the mRAP and right ventricular end diastolic pressure (RVEDP) decreased (although not always to normal), and the CI and mSAP increased. The progression to CS was less frequently observed in patients who underwent TT and PPCI compared to patients who did not receive reperfusion. Without reperfusion therapy (RT) the mortality rate was 27%; with TT the rate was reduced to 13% (*P* < 0.01) and with PPCI to 8.3% (*P* < 0.01),



with significant differences between the two strategies ($P < 0.01$) (Table 6).

Class C: When PPCI was successful, decreased ventricular arrhythmias, mRAP, RVEDP, mPWP and sPAP were found to be associated with an increased CI and mSAP. Without RT, the in-hospital mortality rate was 80%. With TT, the rate was 100%, and with PPCI, the rate was reduced to 44% ($P < 0.01$) (Table 6).

Mortality analysis

To establish the likelihood of mortality, clinical, echocardiographic and hemodynamic variables were tested. When submitted to stepwise logistic regression analysis, age 64-74 years (OR = 5.1; 95%CI: 1.9-14.1, $P < 0.01$), age ≥ 75 years (OR = 24.2; 95%CI: 7.1-66.5, $P < 0.001$), SSP < 100 mmHg (OR = 7.7; 95%CI: 2.4-19.1, $P < 0.001$) and classes B and C (OR = 38.6; 95%CI: 11.5-97.4, $P < 0.01$) were independent, significant predictors of mortality in the multivariate model.

A progressive increase in long-term mortality was noted for all of the RVI patients. At 8 years, without primary reperfusion, the mortality rate was 32% in class A patients, 48% in class B patients and 85% in class C patients (solid lines). When PPCI was successful, the mortality rate at 8 years was lower than previously observed for each RVI classes (21% for A, 38% for B, and 70% for C, $P < 0.001$, dashed lines) (Figure 2).

DISCUSSION

Our findings indicate that there are significant differences in early outcomes and late mortality among the 3 RVI classes. Thus, our discussion for RVI primary reperfusion treatment will focus on the results of reperfusion procedures in each RVI classes.

Class A

This class is the only RVI category in which the following

question can be raised: Which is best for treating RVI: TT, PPCI or neither?^[6] The query was based on the supposition that (1) many RCA occlusions do not result in significant necrosis or RV dysfunction; (2) some thrombolytic studies have suggested little or no benefit in the absence of RVF; and (3) there are no controlled trials in any category of RVI with TT or PPCI^[1-3,11-21].

Nevertheless, more than 40% of class A RVI patients presented at least one major in-hospital complication. Clinical and life threatening risks of an initially hemodynamically silent RVI can not be dismissed when considering timely RT. Although both reperfusion procedures decreased in-hospital mortality in A class RVI patients, our findings suggest that all patients with a hemodynamically silent RVI should undergo PPCI (when available).

Class B

Successful reperfusion of the RCA was associated with near normalization of the mRAP and RVEDP, improvement in the CO and mSAP and reversal of RVWMA/RVF in 78% and 83%. For this category of RVI patients our results demonstrate superior outcomes using PPCI over TT, based on following observations: (1) TIMI 3 RCA flow was obtained with TT in 64% and with PPCI in 91% of the patients; (2) more major complications such as AVB and ventricular arrhythmias, were observed using TT; and (3) mortality was lower in the PPCI treated patients compared to the TT-treated patients (13% *vs* 9%, $P < 0.01$).

Class C

The higher mortality in CS patients resulted from the substantial myocardial damage reflected by the more severe abnormalities in the RV hemodynamic measurements compared to those for class B patients. These findings are also consistent with the subset of patients who were likely to have suffered concomitant severe RA and RV ischemic dysfunction and, as expected, presented with major cardiac complications^[2,3,14,15,22,23].

However, with PPCI we demonstrated a significant reduction in mortality (44%) due to (1) restored perfusion of the RCA and its major branches (TIMI 3 flow: 87%) and reversal of RVWMA (69%); (2) reduced mRAP, RVEDP and mPWP and increased CO and mSAP; and (3) a significant decrease in ventricular arrhythmias.

The mortality with PPCI in our study was lower than that in the SHOCK Registry^[14] (53%), but was higher compared to the study by Brodie *et al.*^[15] in 30 patients (23%). When we compared Brodie's^[15] results with ours, there are close similarities in age, door-to-balloon time, IABP support, stent use and TIMI grade 3-flow after PPCI. However, two important differences were noted. Although twice as many of our patients were sent for PPCI and could make our population more representative of RVI shock patients, the time from the first symptom to reperfusion was twice as long as Brodie's^[15] study.

Prognosis

In our study, we found that the long-term mortality rate

continued to increase after the first year and was different for the 3 RVI classes^[4,11,24-27]. The differences in long-term survival after RVI in the 3 classes in our study could be due to coronary artery disease progression to complete RCA obstruction, significant LAD disease and/or poorly developed collateral coronary circulation, conditions that are most frequently observed in class B and C RVI patients. Perhaps the most important factors affecting outcome were the success or failure of the RT^[1,3,12,16-18].

A study limitation

The major limitation; this analysis used nonrandomized, prospective surveillance and retrospective analysis; thus identified and unidentified confounders may have influenced the trends in reperfusion over time and clinical outcomes. Therefore, it is only an observational study. Nevertheless, this study reports results of RT over time in a large number of patients in 3 RVI categories and the information should be useful in current clinical practice.

In conclusion, PPCI seems to be superior to TT and reduces short and long-term mortality for all 3 RVI categories. Patients in CS should be encouraged to undergo PPCI rather than TT, as a primary reperfusion procedure; consequently, these patients should be transferred to a primary coronary intervention center to decrease the high morbidity and mortality of RVI class C patients.

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COMMENTS

Background

Right ventricular infarction (RVI) is relatively common in patients with acute inferior-posterior left ventricular myocardial infarction. RVI can depress right ventricular (RV) function, resulting in right ventricular failure (RVF) or cardiogenic shock (CS). There are scarce and somewhat conflicting clinical data concerning the effects of interventions designed to achieve reperfusion of the RV myocardium in acute ischemia.

Research frontiers

Several investigators have suggested that RV function improves only after successful thrombolytic therapy (TT), whereas others have reported recovery in the absence of early or even any reperfusion of the right coronary artery (RCA). In a study involving limited number of patients, rapid hemodynamic improvement and excellent clinical outcomes have been reported after successful primary percutaneous coronary intervention (PPCI) of the RCA and its major RV branches. Authors demonstrate that PPCI seems to be superior to TT and reduces short and long-term mortality for all 3 RVI categories. Patients in CS should be encouraged to undergo PPCI rather than TT, as a primary reperfusion procedure.

Innovations and breakthroughs

The findings indicate that there are significant differences in early outcomes and late mortality among the 3 RVI classes. Two important differences were noted. Although twice as many of our patients were sent for PPCI and could make the authors population more representative of RVI shock patients.

Applications

They found that the most important factors affecting outcome were the success or failure of the reperfusion. PPCI seems to be superior to TT and reduces short and long-term mortality for all 3 RVI categories. Patients in CS should be encouraged to undergo PPCI rather than TT, as a primary reperfusion procedure; consequently, these patients should be transferred to a primary coronary inter-

vention (PCI) center to decrease the high morbidity and mortality. The authors consider that their findings must be taking into consideration to be included in treatment guidelines for the RVI.

Terminology

The diagnosis of RVF was based on clinical features, including persistent systemic hypotension (systemic systolic pressure ≤ 100 mmHg, right-sided S₃ and S₄) without features of shock, echocardiographic evidence of ischemic RVF (RV wall motion abnormalities associated with gross RV dilatation), findings that suggest globally depressed RV function and invasive hemodynamic monitoring identifying RVI by a combination of findings that suggest RV dysfunction (low cardiac output and a disproportionate elevation of the mean right atrial pressure compared to the mean pulmonary wedge pressure).

Peer review

This study investigated the impact of reperfusion therapy by means of primary PCI on clinical outcomes in acute RV infarction comparing with TT. The authors concluded that primary PCI is superior to TT and reduces the short-and-long-term mortality. The results are interesting and provide important impact on clinical practice.

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Occurrence of longitudinal stent compression before stent deployment: Two case studies

Adel Aminian, Jacques Lalmand, Dariouch Dolatabadi

Adel Aminian, Jacques Lalmand, Dariouch Dolatabadi, Division of Cardiology, Centre Hospitalier Universitaire de Charleroi, 6000 Charleroi, Belgium

Author contributions: Aminian A wrote the letter; Lalmand J and Dolatabadi D revised the letter for critical intellectual content.

Correspondence to: Adel Aminian, MD, Division of Cardiology, Centre Hospitalier Universitaire de Charleroi, Bd Paul Janson 92, 6000 Charleroi, Belgium. adaminian@hotmail.com

Telephone: +32-71-922217 Fax: +32-71-921147

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Abstract

Several recent reports have described the occurrence of longitudinal stent deformation (LSD, defined as the distortion or shortening of a stent along the longitudinal axis), following its successful deployment. However, few reports have described LSD prior to any stent deployment. This previously unrecognized complication is the result of modifications to stent design. It has been noted that the new-generation stent platforms have a reduced number of connectors, which in turn causes a reduction in longitudinal stent strength. To corroborate previous findings by our lab and others (Vijayvergiya *et al*, 2013), we describe here two cases of LSD prior to stent deployment that occurred due to crushing of the proximal stent edge by the guide catheter while attempting to withdraw the crimped stent. In addition, we discuss the associated risk factors, such as the length of the stent, and specific management strategies, including technical guidelines and use of fluoroscopic guidance for maneuvering the stent during the procedure.

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Key words: Longitudinal stent deformation; Longitudinal stent compression; Stent structure; Percutaneous

coronary intervention; Complication

Core tip: We describe here two cases of longitudinal stent deformation before deployment. This report corroborates the findings previously made by us and others (Vijayvergiya *et al*, 2013) and emphasizes the risk of physical distortion of the stent prior to deployment. We also discuss specific risk factors of and management strategies for this unusual complication.

Aminian A, Lalmand J, Dolatabadi D. Occurrence of longitudinal stent compression before stent deployment: Two case studies. *World J Cardiol* 2014; 6(1): 23-25 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i1/23.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i1.23>

TO THE EDITOR

The case study published by Vijayvergiya *et al*^[1] on longitudinal stent deformation (LSD) has been of great interest to us. The authors describe two cases of proximal LSD involving Promus Element stents that occurred before stent deployment. In both cases, the stent deformations were due to crushing of the proximal stent edge by the guide catheter that occurred while attempts were made to withdraw the crimped stent. Our group was the first to report a similar case involving a non-deployed 3.5 mm × 38 mm Taxus Element stent^[2]. Since then, we have encountered two additional cases of LSD involving non-deployed stents.

The first case was a 69-year-old woman, who was required to undergo elective percutaneous coronary intervention (PCI) of the proximal and distal right coronary artery (RCA). Access was obtained *via* the right radial artery using a 6.5 Fr sheathless JR4 guide catheter (Asahi Intecc Co, Japan). Extensive guidewire-induced dissection of the RCA led to complications in the procedure. A 3.5 mm × 38 mm Multi-Link 8 stent (Abbott Vascular,

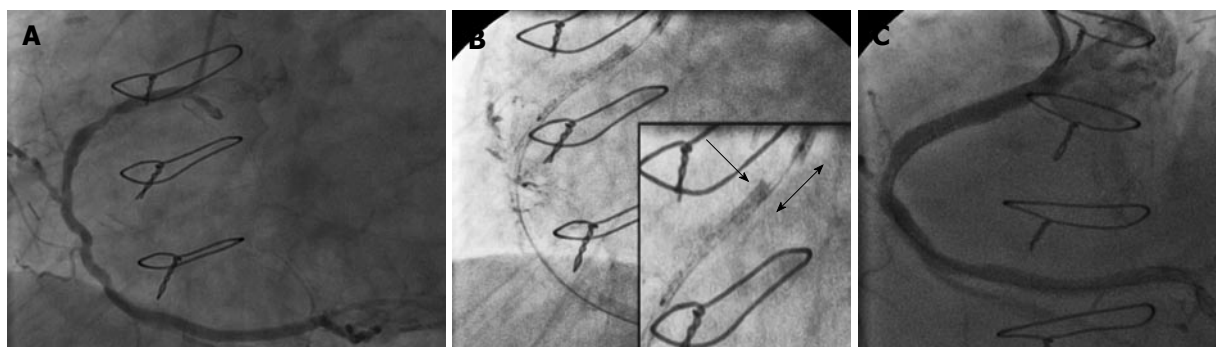


Figure 1 Longitudinal stent deformation of a non-deployed stent in a 81-year-old woman. A: Coronary angiogram showing a long, severe and calcified stenosis involving the proximal and the mid-section of the right coronary artery; B: Severe longitudinal compression of a non-deployed 3.5 mm \times 28 mm Multi-Link stent by the guide catheter, resulting in an 8-10 mm shortening in stent length and an accorded aspect of the proximal stent edge; C: Placement of a 3 mm \times 26 mm Integrity stent for residual lesion coverage. The final angiographic result is shown.

United States) and a 3 mm \times 38 mm Promus Element stent (Boston Scientific, United States) were placed in the proximal and mid-section of the RCA, respectively. Angiographic control showed the presence of residual dissection in the distal RCA. An attempt was made to place a 2.75 mm \times 38 mm Promus Element stent in the distal RCA, but it failed to pass the mid-RCA. While withdrawing the crimped stent, deep engagement of the sheathless catheter occurred, and the proximal edge of the stent became blocked at the distal tip of the catheter. Further attempts to capture the crimped stent resulted in significant compression of the proximal stent edge. Hence, the operator decided to deploy the stent in the proximal RCA on a previously implanted stent. After performing serial high-pressure post-dilatation with a 3.75 mm non-compliant balloon and with the help of a guide catheter extension, a 3 mm \times 28 mm Xience Prime stent (Abbott Vascular) was successfully deployed in the distal RCA. Notably, the patient recovered well and remained asymptomatic throughout the 20-mo clinical follow-up.

The second case was an 81-year-old woman, who was required to undergo elective PCI of the proximal and mid-RCA (Figure 1A). Access was obtained *via* the right femoral artery using a 6 Fr JR4 guide catheter. After performing serial predilatations with a 3 mm balloon, an attempt was made to place a 3.5 mm \times 28 mm Multi-Link stent (Abbott Vascular) but it failed to pass the mid-lesion. While withdrawing the crimped stent, the guide catheter was pulled in and the proximal RCA was engaged. Concomitantly, the proximal edge of the stent was blocked at the distal tip of the guide catheter. After several attempts were made to pull the crimped stent into the guide catheter, it became angiographically evident that the guide catheter had crushed the proximal edge of the stent while the supporting balloon had partially entered the guiding catheter. As a result, the stent was shortened in length by approximately 8-10 mm before being deployed and had an accorded aspect at the proximal stent edge (Figure 1B). It was decided to deploy the stent in the proximal RCA and to cover the mid-section with a second stent. Post-dilatation was performed at high-pressure with a 3.5 mm non-compliant balloon and a 3 mm

\times 26 mm Integrity stent (Medtronic, United States) was placed on the residual mid-lesion. The final angiographic result was deemed acceptable (Figure 1C). The patient recovered well and remained asymptomatic throughout the 15-mo clinical follow-up.

Mamas *et al.*^[3] defined LSD as the distortion or shortening of a stent along the longitudinal axis following its successful deployment. We have reviewed the collective findings from the two cases reported by Vijayvergiya *et al.*^[1] and the three cases from our center, where LSD occurred prior to stent deployment. In all cases, the crushing of the proximal stent edge by the guide catheter during withdrawal of the balloon-stent system caused the LSD. A second important observation is that stent deformation led to an inability to recapture the crimped stent into the guide catheter and severely limited balloon-stent maneuverability. This difficulty arose in certain complex cases, mainly involving the Element Platform (4 out of 5 cases) and the Multi-Link Platform (1 case). Since all five cases utilized a long stent (38 mm long in 4 cases, and 28 mm long in 1 case), stent length could be an important risk factor in causing this unusual complication. Fluoroscopic images showed a clear separation between the proximal balloon marker and the proximal stent edge in all cases, making the diagnosis quick and conclusive. This case series confirms previous findings and highlights the risk of occurrence of LSD prior to stent deployment. The mechanism of the mishap reported here seems consistent with previous reports and involves a reduction in longitudinal stent strength frequently noted with newer stent-platforms.

Based on these reports, we recommend that in cases where withdrawal of a non-deployed new-generation stent into the guide catheter is difficult, the stent should be maneuvered carefully under fluoroscopic guidance. It is imperative to keep the guide catheter and the crimped stent in parallel axes. If resistance persists, advancement and careful rotation of the crimped stent could be attempted before its removal. When proximal stent deformation is visible or if a gap appears between the proximal balloon marker and the proximal stent edge, we strongly advocate avoiding forceful removal of the crimped stent, as it could worsen the stent deformation. In such cases,

the operator has the option to either inflate the stent in another non-consequential location, or to pull back the stent and the guide catheter together. However, the latter strategy risks either loss of the coronary guidewire, which could be inconvenient in specific settings, such as dissections, or the previously reported difficulty in guidewire crossing through lesions. When choosing to deploy the deformed stent, efforts should be made to post-dilate the stent to avoid under-expansion and malapposition, which can lead to stent restenosis and thrombosis. We also recommend avoiding, as much as possible, deep intubation of the guide catheter during stent withdrawal, although sometimes this is unavoidable.

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No. 62 Dongsihuan Zhonglu, Chaoyang District,

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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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Statistical expression

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Contents

Monthly Volume 6 Number 2 February 26, 2014

- | | | |
|------------------------|----|---|
| TOPIC HIGHLIGHT | 26 | Hypertrophic cardiomyopathy in 2013: Current speculations and future perspectives
<i>Efthimiadis GK, Pagourelas ED, Gossios T, Zegkos T</i> |
| REVIEW | 38 | The role of nutrition and nutraceutical supplements in the treatment of hypertension
<i>Houston M</i> |
| MINIREVIEWS | 67 | Therapeutic interventions for heart failure with preserved ejection fraction: A summary of current evidence
<i>Asrar ul Haq M, Wong C, Mutha V, Anavekar N, Lim K, Barlis P, Hare DL</i> |
| CASE REPORT | 77 | Infiltrative cardiac lymphoma with tricuspid valve involvement in a young man
<i>Ngow HA, Wan Mohd Nowalid WK</i> |

Contents

World Journal of Cardiology
Volume 6 Number 2 February 26, 2014

APPENDIX I-V Instructions to authors

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World Journal of Cardiology
Room 903, Building D, Ocean International Center,
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Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: bpgoffice@wjgnet.com
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Hypertrophic cardiomyopathy in 2013: Current speculations and future perspectives

Georgios K Efthimiadis, Efstathios D Pagourelas, Thomas Gossios, Thomas Zegkos

Georgios K Efthimiadis, Efstathios D Pagourelas, Thomas Gossios, Thomas Zegkos, Cardiomyopathies Center, First Cardiology Department, AHEPA University Hospital, Medical School, Aristotle University of Thessaloniki, 54622 Thessaloniki, Greece

Author contributions: Efthimiadis GK and Pagourelas ED reviewed the literature, organized and wrote the various sections of the paper; Gossios T contributed to the manuscript's format and together with Zegkos T critically reviewed and edited the final version of this paper.

Correspondence to: Georgios K Efthimiadis, MD, Cardiomyopathies Center, First Cardiology Department, AHEPA University Hospital, Medical School, Aristotle University of Thessaloniki, 1 Al Svolou str, 54622 Thessaloniki, Greece. efthymos@med.auth.gr
Telephone: +30-231-994820 Fax: +30-231-994820

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Abstract

Hypertrophic cardiomyopathy (HCM), the most variable cardiac disease in terms of phenotypic presentation and clinical outcome, represents the most common inherited cardiomyopathic process with an autosomal dominant trait of inheritance. To date, more than 1400 mutations of myofilament proteins associated with the disease have been identified, most of them "private" ones. This striking allelic and locus heterogeneity of the disease certainly complicates the establishment of phenotype-genotype correlations. Additionally, topics pertaining to patients' everyday lives, such as sudden cardiac death (SCD) risk stratification and prevention, along with disease prognosis, are grossly related to the genetic variation of HCM. This review incorporates contemporary research findings and addresses major aspects of HCM, including preclinical diagnosis, genetic analysis, left ventricular outflow tract obstruction and SCD. More specifically, the spectrum of genetic analysis, the selection of the best method for obstruction alleviation and the need for a unique and accurate

factor for SCD risk stratification are only some of the controversial HCM issues discussed. Additionally, future perspectives concerning HCM and myocardial ischemia, as well as atrial fibrillation, are discussed. Rather than enumerating clinical studies and guidelines, challenging problems concerning the disease are critically appraised by this review, highlighting current speculations and recommending future directions.

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Key words: Hypertrophic cardiomyopathy; Preclinical diagnosis; Left ventricular outflow obstruction; Sudden cardiac death; Genetic analysis

Core tip: Hypertrophic cardiomyopathy (HCM) represents the most common inherited cardiomyopathic process with an autosomal dominant trait of inheritance. This review incorporates contemporary research findings and addresses major and controversial aspects of HCM, including preclinical diagnosis, genetic analysis, left ventricular outflow tract obstruction, sudden cardiac death, myocardial ischemia and atrial fibrillation. Rather than enumerating clinical studies and guidelines, challenging problems concerning the disease are critically appraised by this review, highlighting current speculations and recommending future directions.

Efthimiadis GK, Pagourelas ED, Gossios T, Zegkos T. Hypertrophic cardiomyopathy in 2013: Current speculations and future perspectives. *World J Cardiol* 2014; 6(2): 26-37 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i2/26.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i2.26>

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) represents the

most common inherited cardiac disease, affecting 1 in every 500 people in the general population^[1,2]. Classically, it is defined by the presence of a hypertrophied, non-dilated left ventricle (LV) in the absence of any cause capable of producing the magnitude of evident hypertrophy, such as pressure overload or storage/infiltrative diseases^[3,4]. The main features of the disease are: (1) clinical and genetic heterogeneity, altering phenotypic expression and complicating both clinical and preclinical diagnosis; (2) obstruction, either in the left ventricular outflow tract (LVOTO) or in the midventricular level (MVO), and their pathophysiological significance; and (3) sudden cardiac death (SCD) and risk factors predisposing to it. Evaluation of the mentioned characteristics is essential in the assessment of every patient with HCM.

In this context, the aim of this review is to critically present current knowledge concerning the most controversial fields of HCM, including preclinical diagnosis, obstruction and SCD, and to briefly discuss treatment modalities that might prove useful, especially when applied in the preclinical level. Rather than enumerating clinical studies and guidelines, the authors have tried to appraise challenging problems concerning the disease, highlight current speculations and recommend future directions.

PRECLINICAL DIAGNOSIS

According to current guidelines, HCM diagnosis is mainly based on the detection [either by echocardiography or magnetic resonance imaging (MRI)] of a maximal wall thickness ≥ 15 mm or on the presence of a mild hypertrophy (13-14 mm) coexisting with a positive family history of HCM and/or an HCM compatible ECG^[3-5]. Although diagnosis in cases of overt hypertrophy seems simplified, with the clinical distinction and differentiation of phenocopies being rather challenging, the real challenge in terms of HCM diagnostic evaluation today is preclinical diagnosis.

Preclinical diagnosis refers to the detection of subjects that carry any HCM-causing gene mutation, before or even without the development of LV hypertrophy [genotype (+)/phenotype (-) subjects]. The concept that HCM pathology may exist in the absence of LV hypertrophy is quite old^[6], but the ability to recognize the presence of early myocardial changes is quite new. Although genetic testing may become the ultimate tool for assessing the risk of disease development, several issues complicate its use as a screening tool.

In 60% of cases, HCM is a familial disease with an autosomal dominant trait of inheritance. To date, more than 1400 HCM-related mutations in genes encoding different sarcomere or non-sarcomere proteins have been identified^[4]. Among them, definitive HCM causative mutations are those implicating 8 sarcomere genes with approximately 80% of identified mutations concerning cardiac β -myosin heavy chain and cardiac myosin binding protein C^[7-18]. Apart from the great number of mutations recognized up to now, some genetic defects, especially

those concerning the cardiac myosin binding protein C gene, are founding mutations and referred to as homogeneous and closed concentrated populations. To further complicate things, the latest studies have documented that 5% of HCM families carry 2^[19-21] or even 3 distinct causative mutations^[22], including homozygous and double or compound heterozygous mutations. The “privacy” of many mutations (unique genetic defects inside specific families), the variable penetrance of recorded mutations allowing various phenotypic severity, the complexity of distinction between a genetic polymorphism and a causative mutation, and the involvement of multiple potential disease modifying variants^[23] have led to a decrease of initial enthusiasm about the utility of genetic analysis in preclinical diagnosis. While still in search of the “Holy Grail” which is the phenotype-genotype correlation, the utility of genetic analysis is confined mostly to identify the proband’s relatives sharing the mutation and to diagnose HCM phenocopies, such as Anderson-Fabry’s disease and other glycogen or lysosomal storage diseases.

The complexity of genetic analysis has led to the adoption of other diagnostic approaches to unveil HCM in the preclinical stage, mainly by discovering disease features that precede the development of overt hypertrophy. In the excellent paper by Geisterfer-Lowrance *et al.*^[7], based on a mouse model of familial HCM, cardiac dysfunction preceded histopathological changes, myocyte disarray came next, while hypertrophy and fibrosis tended to increase with age (Figure 1). Reflecting on this experiment in clinical settings, cardiac dysfunction (detected by tissue Doppler imaging), myocyte disarray (encoded by new ECG abnormalities), hypertrophy (visualised by echocardiography or cardiac MRI) and fibrosis [detected by cardiac MRI Late Gadolinium Enhancement (LGE)] are early signs of HCM that should be properly searched for (Figure 2)^[24-26]. Overall, several clinical reports have demonstrated that the majority of HCM genotype (+)/phenotype (-) subjects display “early” myocardial functional or histopathological changes, such as reduced tissue Doppler imaging-derived systolic and diastolic velocities, abnormal ECG, cardiac magnetic resonance (CMR)-visualized myocardial crypts, mitral leaflet elongation and evidence of a fibrotic state, such as increased type I procollagen synthesis, CMR-increased myocardial extracellular volume, and late gadolinium myocardial enhancement^[24-27].

Preclinical diagnosis of HCM has many medical and social implications. At present, there is no evidence that early detection will change the course of the disease; however, early application of therapy may improve the lifelong management of these subjects. Experimental therapies in HCM-models using conventional medications have shown promising results on reversal or prevention of hypertrophy and fibrosis. Larger studies in clinical settings during the preclinical stage of HCM are necessary to demonstrate the potential benefit in prevention of HCM phenotypic changes or complications, including SD.

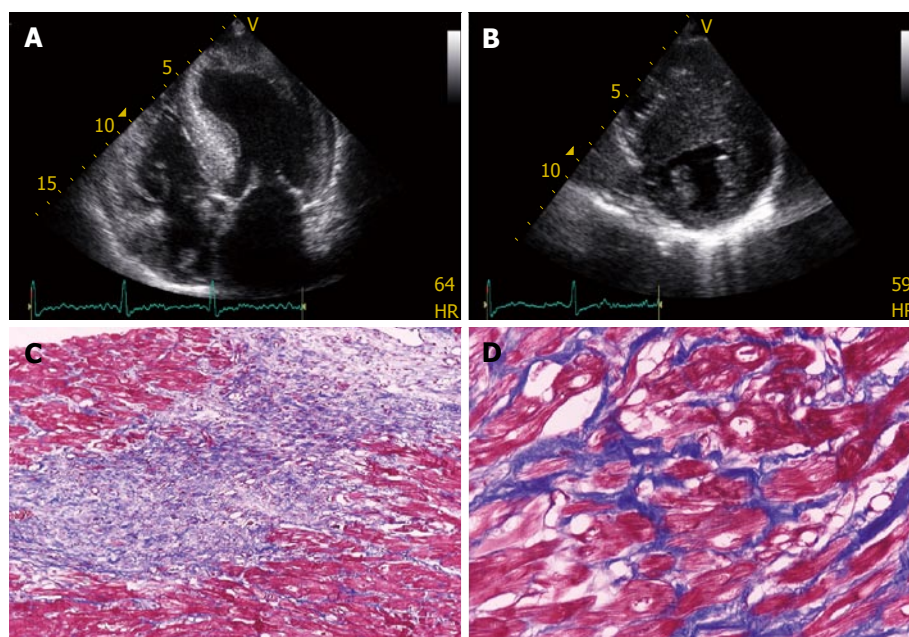


Figure 1 Echocardiographical and pathological features of hypertrophic cardiomyopathy. A: An apical 4-chamber view of a patient with hypertrophic cardiomyopathy showing a hypertrophied, non-dilated left ventricle; B: Excessive thickness of interventricular septum (eccentric hypertrophy) is also optimally visualized from parasternal short axis views; C: Myocardial disarray and extensive fibrosis ($\times 10$ Trichrome Masson); D: Myocardial disarray and interstitial fibrosis ($\times 40$ Trichrome Masson).

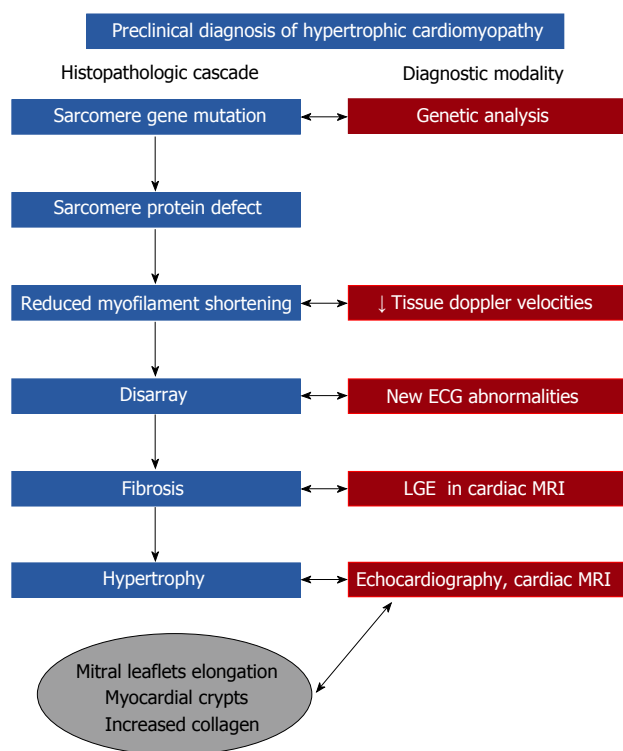


Figure 2 Preclinical diagnosis in hypertrophic cardiomyopathy. The figure shows histopathological cascade of the disease and diagnostic modalities used to detect abnormalities in each stage. LGE: Late gadolinium enhancement; MRI: Magnetic resonance imaging.

SIGNIFICANCE AND TREATMENT OF OBSTRUCTION

After a lasting controversy concerning its role in HCM, obstruction is evidenced to be related to severity of symptoms, especially by augmentation of gradient during exercise, in the context of diastolic dysfunction and myocardial ischemia^[28]. Maron *et al.*^[29] in 2003 docu-

mented that obstruction at rest is a strong, independent predictor of progression to severe heart failure and death, while according to another study, 70% of patients are echocardiographically found to have obstruction at rest or during exercise^[30]. Despite establishing a connection between LVOTO and progression to heart failure in HCM, a controversy concerning the potential impact of LVOTO on SCD survival is still ongoing. Two major studies have demonstrated that a resting gradient > 30 mmHg was associated with a 2.4-fold increase in the risk of SCD^[29,31], presenting, however, a very low positive predictive value ($< 10\%$) and a very low SCD annual rate (0.37%-1.5%)^[29,31,32]. In our cohort of HCM patients, obstruction did not show a significant correlation with SCD incidence^[33]. At present, obstruction at rest does not serve as a sole risk factor for SCD^[34]. Probably, severe gradients (> 100 mmHg) may serve as SCD arbitrator in the context of other risk factors^[4].

A minority of HCM patients present with a mid-LV level obstruction due to midventricular muscular apposition creating an hourglass-shaped LV^[35-40]. MVO is associated with an unfavorable prognosis in terms of end stage HCM, SCD and lethal arrhythmic events^[40,41]. A very challenging and distinct complication of midventricular obstruction is that of LV apical aneurysm formation associated with transmural myocardial scarring. Maron *et al.*^[42] and our team found a 2% prevalence of apical aneurysms in HCM patient cohorts. About 70% of patients with apical aneurysms had a midventricular, whereas the remaining 30% presented with an apical type of hypertrophy. More than 40% of patients with an apical aneurysm experienced cardiovascular complications, including SCD, appropriate implantable cardioverter-defibrillator (ICD) discharges, thromboembolic stroke and progressive heart failure-death, over a 4 years mean time of follow-up^[42].

Concerning therapeutic approaches for obstruction, interventional procedures should be applied in patients

who are severely symptomatic, with a maximal instantaneous gradient > 50 mmHg at rest or with physiological provocation despite optimal medical treatment (B-blockers, verapamil, disopyramide or combination thereof)^[4]. The major goal of pharmacological therapy in symptomatic patients with HCM is to alleviate symptoms of exertional dyspnea, palpitations and chest discomfort, which may reflect pathophysiological mechanisms such as LVOTO obstruction, reduced supply of myocardial oxygen, mitral regurgitation and impaired LV diastolic relaxation and compliance^[3,43]. Beta blockers accomplish that through their negative inotropic and chronotropic effects^[44] improving myocardial oxygen supply-demand relationships, prolonging the diastolic filling period, allowing for more efficient inactivation of myocardial contractile proteins and leading thus to LVOTO alleviation^[45,46]. Negative inotropic and rate lowering effects are the mechanism of action for verapamil and diltiazem, whereas negative inotropic action is also the pharmacological pathway of disopyramide^[4].

Surgical septal myectomy, accomplished through a transaortic approach and extended muscular resection (Morrow procedure), resulting in physical enlargement of the LV outflow has been fairly considered the gold standard of invasive therapies for relief of obstruction in severely symptomatic HCM patients^[47-54]. In a major retrospective study from the Mayo Clinic, surgical myectomy performed in severely symptomatic patients with obstructive HCM was associated with a long term survival equivalent to that of the US general population and superior to survival observed in patients with obstructive HCM without operation^[50]. In another study coming from the same center, surgical myectomy in patients carrying an ICD was associated with a significant reduction in the rate of appropriate ICD discharge and a reduction in the risk of SCD^[52].

Alcohol septal ablation *via* a percutaneous intracoronary approach uses administration of absolute ethanol to the septal perforator branch, inducing a localized infarction of the basal septum at the point of contact of the anterior mitral valve leaflet, reducing thereby the LV outflow tract gradient^[53-55].

Selection of the best interventional treatment should depend on demographic, anatomic-electrical and hemodynamic criteria. More specifically, the age of the patient, operator and institutional experience on each specific method, the presence of comorbidities (chronic kidney disease, coronary artery disease, chronic pulmonary or hepatic impairment, *etc.*) and, last but not least, patient's preference, are among the most crucial demographic factors influencing implementation of an interventional strategy. Additionally, the magnitude and extent of ventricular hypertrophy, dislocation of papillary muscles and their functionality, the presence of intrinsic mitral valve disease potentially demanding the need of additional surgical approaches, the complexity of coronary vasculature along with the existence or not of conduction abnormalities may influence final decisions based on an electro-anatomic and hemodynamic basis^[4].

Although long-term outcome studies comparing the effectiveness and mortality after alcohol septal ablation or septal myectomy are lacking, a recent meta-analysis reviewing 12 studies comparing the two interventional techniques found no significant differences concerning short and post-adjustment long term mortality, post-intervention functional status, improvement in New York Heart Association functional class, ventricular arrhythmia occurrence, re-interventions performed and post-procedure mitral regurgitation^[56]. However, septal ablation was connected to a higher post-procedure incidence of complete heart block requiring a permanent pacemaker (10%-20% *vs* 2% after surgery)^[4], while it was found to increase the risk of right bundle branch block (RBBB). Patients with left bundle branch block and RBBB are more likely to develop complete heart block with surgery and alcohol septal ablation, respectively^[4]. Finally, the percentage of patients showing a higher residual gradient was also deemed to be higher among patients having undergone septal ablation^[56].

Current evidence suggests that any attempt to conduct a double blind randomized study comparing the long term effects of the 2 main therapeutic options for LVOTO in HCM would be complicated, if not impossible. Furthermore, septal ablation and septal myectomy are 2 very different techniques; the former causing ischemia and generating a scar and the latter leading to myocardial resection. The myocardial scar caused by septal ablation has aroused concern of a potentially increased risk of malignant arrhythmias. Ventricular arrhythmias have been reported as an effect of ischemia in the early post procedural phase^[57,58]. However, no increased risk of malignant arrhythmias has been shown in patients who already had an ICD implanted because of a previously estimated high risk of SCD^[59,60]. In a recent report, various factors, including age ≥ 65 years, gradient < 100 mmHg, septal hypertrophy ≤ 18 mm and left anterior descending artery diameter < 4.0 mm, were the strongest patient characteristics that predicted clinical success after septal alcohol ablation^[61]. The increasing experience of involved tertiary centers and proper training of physicians providing both interventional treatments will diminish the rate of complications in future and significantly alter the natural course of the disease, especially among those patients presenting with more symptoms and eventually higher mortality.

RISK AND PREVENTION OF SCD

SCD is the most dramatic complication of HCM. Even although primary estimates of the SCD rate emanating from tertiary center based cohorts have been as high as 6% per year, true prevalence based on data coming from large scale community registries is significantly lower, approximately 0.7% annually^[3,4]. It is evident that the prevalence of SCD is higher in younger people, approximately before 35 years of age, even although according to other studies, longevity is not synonymous with immunity^[62,63]. HCM related SCD is the leading mortality cause among

Table 1 Risk factors for sudden cardiac death in hypertrophic cardiomyopathy

Established risk markers	Risk modifiers or novel risk factors
Prior resuscitated cardiac arrest (VF, sustained VT)	LGE in MRI
MWT > 30 mm	Marked LVOTO
FH of SCD	Severe or multiple sarcomeric mutations
Syncope	Certain phenotypic expressions: Apical aneurysms, midventricular obstruction
NSVT	Severe systolic or diastolic impairment, <i>e.g.</i> , burnt out HCM, restrictive pattern
ABPR	CAD
	Arrhythmic substrate: Atrial fibrillation

VF: Ventricular Fibrillation; VT: Ventricular tachycardia; MWT: Maximum Wall thickness; FH: Family history; SCD: Sudden cardiac death; NSVT: Non sustained ventricular tachycardia; ABPR: Abnormal blood pressure response to exercise; LGE: Late Gadolinium Enhancement; MRI: Magnetic resonance imaging; LVOTO: Left ventricular outflow tract obstruction; CAD: Coronary artery disease; HCM: Hypertrophic cardiomyopathy.

competitive athletes following different sport disciplines^[64,65]. The vast majority of SD (85%) occurs during daily activities (walking, rest, driving or during sleep), while 70% of patients dying suddenly are asymptomatic or have few symptoms (functional class I or II)^[62]. Despite the fact that SCD objectively affects a small minority of HCM patients, early recognition of predisposing factors and concomitant prevention still remains a major clinical challenge since SCD and associated lethal arrhythmic events may be fully prevented, either primarily or secondarily, by means of implantable ICDs.

Apart from personal history of ventricular fibrillation (VF), sustained ventricular tachycardia (SVT) or resuscitated cardiac arrest which has been found to represent the highest risk predisposing to new potentially lethal arrhythmic events (secondary prevention)^[66-68], 5 non interventional clinical factors have been identified up to now to represent risk markers for SCD in HCM: (1) Family history of SCD affecting at least one first degree relative < 40 years; (2) Syncope, without a known causal factor occurring in the recent past (< 6 mo); (3) Extreme left ventricular hypertrophy as this is represented by a maximum wall thickness of any myocardial segment > 30 mm; (4) Abnormal blood pressure response (ABPR) to exercise, defined as either a failure of systolic blood pressure to increase by at least 20 mmHg or a drop below baseline resting values during effort and even a drop of systolic pressure during maximal exercise; and (5) Non SVT (NSVT), defined as recording on ambulatory 24-h Holter of ≥ 3 consecutive ventricular ectopic beats at a rate of ≥ 120 beats lasting < 30 s^[69-73]. NSVT is considered a risk factor for SCD, primarily in patients under the age of 30^[74].

Recent HCM guidelines have suggested an escalation in risk stratification, suggesting that personal history of SVT or VF is Class I indication for ICD implantation^[4].

Existing literature suggests that these patients have 33% mortality in 7 years^[66] and that in 5 years 41% will experience SD or ICD-discharge^[67]. The presence of a family history of SCD, syncope or a maximal wall thickness > 30 mm confers a Class IIa indication for ICDs, whereas NSVT or ABPR alone probably could not justify ICD implantation needing reassessment of risk profile based on the rest of risk factors or potential arbitrators^[4]. Several clinical or laboratory aspects of HCM have been studied as potential risk modifiers for SCD, as shown in Table 1. Among them, 3 certain features of HCM may affect our decision in favor of ICD implantation based on evidence from published trials^[4,69]: the presence of LGE on MRI^[75]; certain mutations, especially coexistence of more than 1 sarcomere mutation^[22]; and marked LV outflow tract obstruction at rest^[4,29,31].

All of the above mentioned factors describe the very same phenomenon from a different point of view: extent of replacement and interstitial fibrosis leading to different conduction pathways in the myocardium, thus facilitating reentry events and finally malignant ventricular tachyarrhythmias^[76,77]. Based on the previous assumptions, detection of LGE by MRI could be the main pillar of SCD risk stratification since it reflects the extent of fibrosis, the main determinant of malignant arrhythmias. However, a recent meta analysis concluded that LGE showed a trend towards significance for predicting SCD/aborted SCD (pooled OR = 2.39; 95%CI: 0.87-6.58, $P = 0.091$), failing to accurately define individual patients with HCM reaching this end point^[75]. To date, there is no compelling published evidence that the extent is more important than just the presence of LGE for risk-prediction. Moreover, the 2011 current guidelines emphasize that it is the presence and not the extent of LGE that relates to adverse CV events. However, this is an interesting, controversial topic that should be addressed by future research [an ongoing multicenter trial with over 1000 HCM patients will probably show that the extent of LGE is also relevant (Martin Maron, ACC 2013)].

ICDs have proved to be effective in terminating life-threatening ventricular tachyarrhythmias in HCM, altering the natural course of the disease and prolonging life. ICDs should be offered after detailed discussion with the patient and his/her family and after benefits are anticipated to outweigh the potential risks. Data from retrospective analysis of sizeable cohorts of recipients have demonstrated that the number of risk factors prior to implantation for primary prophylaxis is disproportionate to the number and frequency of appropriate shocks delivered, while the time interval from ICD implant to first appropriate device discharge is quite variable in length since some patients have survived over 10 years after an initial episode of cardiac arrest without receiving appropriate ICD discharges^[78,79]. A careful evaluation of data coming from American and European registries could easily reveal that the annual rate of ICD adverse events (including inappropriate shocks and lead complications) may range between 8.6% and 25%, at least 2-fold higher than the rate of patients receiving appropriate shocks per

year^[78-80]. The rate of inappropriate shocks and lead dislodgment/fractures seems to be higher among younger populations (children, adolescents), mainly due to their increased activity levels and body growth^[81].

HCM AND BLUNTED MYOCARDIAL PERFUSION

For much of the past 50 years, HCM progression was mainly connected to LVOTO and diastolic dysfunction, under appreciating (or even worse, under recognizing) myocardial ischemia as an important pathophysiological component of the disease. Even now, assessment of myocardial ischemia is currently not part of routine clinical diagnostic or management strategies in HCM^[4].

Initial evidence that myocardial ischemia participates in the pathophysiological mechanism of HCM was based on post mortem studies of patients who had died suddenly and presented with extensive areas of myocardial damage. A spectrum of ischemic injury was observed, from an acute phase with coagulative necrosis and neutrophilic infiltrate to a chronic post-necrotic replacement-type fibrosis, always in the absence of atherosclerotic epicardial coronary artery disease^[82]. In addition to gross pathological evidence of myocardial scarring, autopsy studies in HCM patients have shown structural abnormalities of intramural coronary arterioles, characterized by thickening of the intima and/or medial layers of the vessel wall associated with a decreased luminal cross-sectional area. These morphological changes are the main substrate for functional decompensation, which translates to blunted myocardial blood flow during stress^[83-85].

Among contemporary non invasive imaging modalities that have been used for revealing impaired myocardial blood flow in HCM, PET with either ¹³N labeled ammonia or ¹⁵O-labeled water is the most reliable^[86]. The measurement of myocardial blood flow under basal conditions and in conditions of near-maximal vasodilatation (after intravenous adenosine or dipyridamole) permits the calculation of coronary flow reserve, that is, the ratio of maximum to basal blood flow. On the other hand, although SPECT myocardial perfusion imaging is widely available, this technique is limited by allowing only the assessment of relative changes in regional perfusion and by an inability to quantify absolute MBF^[87].

Similarly to PET, recent stress CMR studies in HCM showed blunted myocardial blood flow in response to stress. Importantly, areas of the myocardium in which fibrosis was present (as determined by LGE) were most often associated with reduced myocardial blood flow^[88], even although a small proportion of patients had LGE in the absence of perfusion abnormalities^[89]. Taken together, these CMR observations show an association between ischemia, myocardial fibrosis and LV remodeling, providing further support for the principle that abnormal blood flow caused by microvascular dysfunction is responsible for myocardial ischemia-mediated myocyte death and ultimately repair in the form of replacement

fibrosis^[87]. Traditional, noninvasive methods for detecting myocardial ischemia clinically, including ST-segment changes on 12-lead and ambulatory Holter electrocardiographic monitoring or exercise testing, have proved to be insufficiently sensitive or specific for detecting ischemia in HCM^[87].

Verapamil and beta-blockers may improve symptoms of chest pain and exertional dyspnea in HCM. This probably occurs *via* reduction in heart rate and oxygen consumption and possibly because of direct effects on the microvasculature and diastolic filling leading to improved perfusion, especially in the mostly “stressed” subendocardial regions^[87]. Also, since there is now evidence showing an improvement in myocardial perfusion after septal reduction therapy^[90], consideration should be given to these procedures to relieve severe chest pain refractory to drug therapy^[87].

Concerning the importance of revealing blunted myocardial blood flow in HCM, a previous study has shown that severe abnormalities in myocardial blood flow caused by microvascular dysfunction seemed to be a powerful determinant of impaired systolic function, whereas preserved myocardial blood flow identified the low-risk subgroup^[91]. Therefore, an impaired myocardial blood flow could possibly differentiate individuals with a higher risk for progression towards a “burnt out” phenotype (dilatation and severe systolic impairment). This has important clinical implications since HCM patients in the end stage experience a high rate of unfavorable disease consequences, including progressive heart failure (often requiring heart transplantation) and SCD (prompting consideration for prophylactic ICD).

In conclusion, blunted myocardial blood flow seems to be an important component of HCM physiology. However, many current controversies need to be clarified by future research. First of all, the dynamic interaction between fibrosis and ischemia needs further study so as to define which phenomenon precedes the other in the vicious circle set. This is extremely important since identifying the stage when active myocardial ischemia begins (with respect to the development of the HCM phenotype) answers the question of whether impaired myocardial blood flow could be considered an early therapeutic target. Secondly, future research should highlight optimal non-invasive imaging modalities as well as biomarkers with sufficient sensitivity and specificity to reveal HCM patients with ischemia predisposing to disease progression. Finally, future studies should not only discover novel therapies targeting myocardial ischemia in HCM (especially for those patients presenting refractory angina to common medication), but also define the groups of patients who should mostly benefit from anti-ischemic treatment.

AF

Patients with HCM are at increased risk of AF compared with age-matched cohorts, while AF is an important cause of symptoms, morbidity and even mortality in pa-

tients with HCM^[4,62]. The 2011 ACC/AHA guidelines for diagnosis and treatment of HCM recognize the importance of AF for HCM prognosis, extrapolating, however, AF diagnostic and therapeutic options recommended for the general population in HCM patients^[92].

The risk of systemic embolization is high in HCM patients with AF but does not seem to be related to the severity of symptoms^[4,62]. Risk scores that seem to be efficient and therapy guiding in the general population (like CHADS2-VASc) might be less effective in HCM where other risk factors may also play an important role in predisposing to embolic events. LVOTO, SAM and of course the magnitude of LA enlargement (a common morphological feature in many HCM patients) seem to be additional factors that increase the risk for stroke^[4,62]. Even although paroxysmal, persistent or chronic AF followed by a CHADS2-VASc score > 2 is a strong indication for anticoagulation with a vitamin K antagonist^[92], the threshold for AF that warrants anticoagulation remains unresolved. For example, should HCM patients with a sole AF episode receive anticoagulant treatment given the high risk of thromboembolism in HCM? Is a large LA volume or volume index sufficient as a risk factor for a vitamin K antagonist prescription in HCM patients prior to AF occurrence or in AF without the presence of other risk factors? Finally, could aspirin prevent embolic episodes in HCM patients with AF and low CHADS2-VASc score?

Contemporary developments in anticoagulation and rhythm control management in AF warrant a cautious assessment before their application in HCM patients. Unfortunately, few data exist concerning the safety and efficacy of dabigatran or activated X factor inhibitors in HCM. Accordingly, the long-term benefits of radiofrequency ablation *vs* antiarrhythmic drugs in patients with HCM remain to be established. Furthermore, there are no data regarding the efficacy of other class I antiarrhythmic agents, sotalol or dronedarone, in HCM^[4,93]. Overall, AF is an important feature of HCM pathophysiology and disease progression necessitating further research efforts to optimize existing treatment options.

NOVEL TREATMENT POTENTIALS

Five decades following the original description of HCM, there is still a dismal paucity of data supporting pharmacological treatment strategies for this complex disease. By comparison, device-based, percutaneous and surgical treatments of LVOTO obstruction have received significantly greater attention, although rarely in a double blind randomized fashion. This can be regarded as a paradox as only a minority of patients requires surgery or a device, whereas the large majority is treated pharmacologically^[94]. Additionally, few data exist concerning the therapeutic approach of HCM patients without obstruction (1/3 of the HCM population)^[94].

Treatment application in the preclinical phase of HCM may have a beneficial effect, whereas treatment during the mature phase of the disease could be rather

problematic since a possible regression of hypertrophy may lead to LV dilation and reduced EF^[95]. Taking into consideration the pathophysiological cascade of HCM progression, early treatment options could be simply divided into 3 categories: therapies targeting impaired calcium homeostasis and related disorders; drugs blocking the results of neurohumoral response secondary to sarcomere dysfunction; and anti-fibrotic agents.

With the knowledge that altered intracellular Ca²⁺ handling occurs early in disease pathogenesis, diltiazem, an L-type calcium channel blocker, inhibited the development of HCM phenotype when administered to young (pre-hypertrophic) mice carrying a pathogenic myosin heavy chain mutation (α MHC403/+)^[96]. Importantly, treatment initiated after the development of LV hypertrophy was unable to reverse the established phenotype in these animals^[96]. In an observational study enrolling a small number (6 patients) of genotype (+)/phenotype (-) HCM patients, oral daily administration of 240 mg of diltiazem led to normalization of early diastolic and systolic velocities about 8 wk after treatment initiation^[97]. Obviously, data on the actions of diltiazem in preclinical HCM patients are lacking, while an ongoing trial which is expected to terminate in December 2013 is testing the effects of diltiazem in preventing phenotypes in preclinical HCM, *i.e.*, in subjects with identified sarcomere mutation with no overt LVH^[98]. In a similar way, ranolazine is another factor that could possibly interrupt the vicious circle of calcium in preclinical HCM, deterring the establishment of an overt HCM phenotype. The drug is currently used as a metabolic modulator in ischemic heart disease^[99] but further insights suggested the role of this drug as a selective inhibitor of INaL in cardiomyocytes. Tomberli *et al*^[100] demonstrated the potential role of intracellular Na⁺ overload in inducing an altered Ca²⁺ homeostasis in HCM myocardial samples. This mechanism can play an important role in cardiac remodeling in HCM.

Statins, 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors and angiotensin II receptor blockers (ARBs) are demonstrated to inhibit angiotensin II-mediated cardiac hypertrophy^[101,102]. Senthil *et al*^[103] treated 15 pre-hypertrophic β MHC-Q403 rabbits with Atorvastatin, 2.5 mg/kg per day, *vs* a placebo group for 1 year. Rabbits treated with statins did not develop hypertrophy and showed a reduction in both the myocyte cross-sectional area and collagen volume fraction. Similarly, Teekakirikul *et al*^[104] treated pre-hypertrophic α -MHC^{719/+} mice with Losartan for 2 wk prior and during Cyclosporine A induction of hypertrophy which prevented the emergence of hypertrophy, non-myocyte proliferation and fibrosis. Although statins and ARBs seemed to be able to reverse hypertrophy and fibrosis and to prevent the development of the phenotype in HCM animal models, these results were not replicated in clinical trials^[105,106].

The rational for using N-acetylcysteine and spironolactone in HCM comes from the demonstration of the anti-fibrotic effects of the drugs in several human tissues and animal models^[107-113]. However, there are no demonstrations of efficacy of the long term treatment on

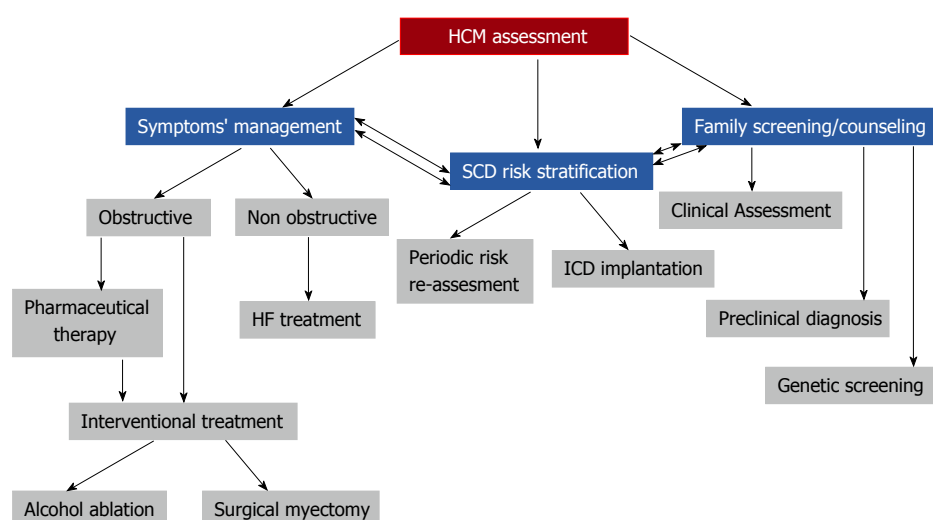


Figure 3 Hypertrophic cardiomyopathy assessment algorithm. A clinician dealing with a HCM patient should face 3 major issues: symptom management based on the existence or not of left ventricular outflow obstruction; sudden cardiac death risk stratification and prevention; and finally, family counseling and advice. HF: Heart failure; SCD: Sudden cardiac death; ICD: Implantable cardioverter defibrillator; HCM: Hypertrophic cardiomyopathy.

humans yet.

CONCLUSION

HCM assessment is based on a multilevel approach taking into consideration symptom (obstruction) management, SCD risk stratification and preclinical diagnosis/genetic screening/counseling (Figure 3). Could diagnostic evaluation and therapeutic approach be substantially improved over the next few years?

There has been a remarkable evolution during the last few years driven by the discovery of new mutations connected with the disease, expanding its known genetic database. Widespread adoption of genetic analysis, at least from tertiary referral centers, involving newer techniques such as next-generation sequencing along with the progress of bio-informatics, will help to better organize genetic bases by faster and more cost effective approaches of the responsible exons, thus bypassing the striking allelic and locus heterogeneity of the disease^[114]. Based on these achievements, differentiating no disease causing polymorphisms from disease causing mutations will become significantly easier, permitting genotype-phenotype correlations from thoroughly followed up patient cohorts. The introduction of proteomics will hopefully facilitate better definition of the molecular mechanisms of the disease, identifying the pathophysiological pathways from genetic mutations to phenotypic presentation and clinical course. All the above developments will certainly highlight new therapeutic targets, which may impede genotypic expression and disease progression, and may provide a more accurate risk assessment for SCD prevention based on an individual clinical-genetic assessment.

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The role of nutrition and nutraceutical supplements in the treatment of hypertension

Mark Houston

Mark Houston, Hypertension Institute, Saint Thomas Medical Plaza, Nashville, TN 37205, United States

Author contributions: Houston M solely contributed to this work.

Correspondence to: Mark Houston, MD, MS, MSc ABAARM, FACP, FAHA, FASH, FACN, FAARM, Hypertension Institute, Saint Thomas Medical Plaza, 4230 Harding Road, Suite 400, Nashville, TN 37205, United States. mhoustonhish@yahoo.com
Telephone: +1-615-2972700 Fax: +1-615-3730302

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Abstract

Vascular biology, endothelial and vascular smooth muscle and cardiac dysfunction play a primary role in the initiation and perpetuation of hypertension, cardiovascular disease and target organ damage. Nutrient-gene interactions and epigenetics are predominant factors in promoting beneficial or detrimental effects in cardiovascular health and hypertension. Macronutrients and micronutrients can prevent, control and treat hypertension through numerous mechanisms related to vascular biology. Oxidative stress, inflammation and autoimmune dysfunction initiate and propagate hypertension and cardiovascular disease. There is a role for the selected use of single and component nutraceutical supplements, vitamins, antioxidants and minerals in the treatment of hypertension based on scientifically controlled studies which complement optimal nutrition, coupled with other lifestyle modifications.

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Key words: Hypertension; Nutrition; Nutritional supplements; Cardiovascular disease; Vascular biology

Core tip: Vascular biology and endothelial dysfunction play a primary roles in hypertension and subsequent

cardiovascular disease. Micronutrients, macronutrients and optimal nutrition and nutritional supplements can prevent, control and treat hypertension through numerous mechanisms related to vascular biology. These treatments are complementary to drug therapy. Oxidative stress, inflammation and autoimmune dysfunction initiate and propagate hypertension and cardiovascular disease. There is a role for the selected use of single and component nutraceutical supplements, vitamins, antioxidants and minerals in the treatment of hypertension based on scientifically controlled studies which complement optimal nutrition, coupled with other lifestyle modifications.

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INTRODUCTION

Vascular disease is a balance between vascular injury and repair (Figure 1). The endothelium is in a strategic location between the blood and the vascular smooth muscle and secretes various substances to maintain vascular homeostasis and health (Figures 2 and 3). Various insults that damage the endothelium, lead to endothelial dysfunction (ED) and may induce hypertension and other cardiovascular diseases. Hypertension may be a hemodynamic marker of injured endothelium and vascular smooth muscle related to finite responses of inflammation, oxidative stress and immune dysfunction of the arteries leading to ED, vascular and cardiac smooth muscle dysfunction, loss of arterial elasticity with reduced arterial compliance and increased systemic vascular resistance. Hypertension is a consequence of the interaction of genetics and environment. Macronutrients and micronutrients are crucial in the regulation of blood pressure (BP) and subsequent

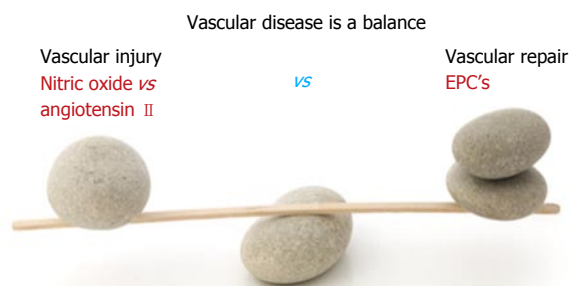


Figure 1 Vascular health is a balance of injury and repair. EPC's: Endothelial progenitor cells.

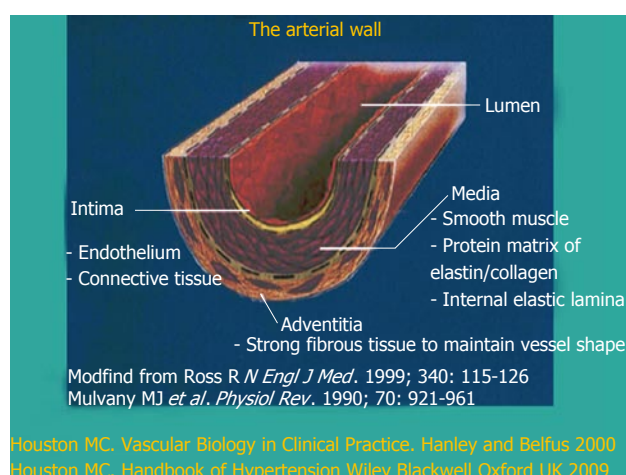
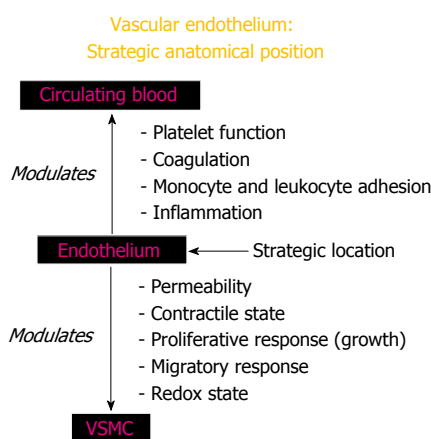


Figure 2 The blood vessel structure.



Vascular Biology in Clinical Practice, Oct. 2000; Mark C. Houston, MD

Figure 3 The role of the vascular endothelium to maintain vascular homeostasis and health. VSMC: Vascular smooth muscle cells.

target organ damage (TOD). Nutrient-gene interactions, subsequent gene expression, epigenetics, oxidative stress, inflammation and autoimmune vascular dysfunction have positive or negative influences on vascular biology in humans. Endothelial activation with ED and vascular smooth muscle dysfunction (VSMD) initiate and perpetuate essential hypertension.

Macronutrient and micronutrient deficiencies are very common in the general population and may be even

more common in patients with hypertension and cardiovascular disease due to genetics, environmental causes and prescription drug use. These deficiencies will have an enormous impact on present and future cardiovascular health outcomes such as hypertension, myocardial infarction (MI), stroke and renal disease. The diagnosis and treatment of these nutrient deficiencies will reduce BP and improve vascular health, ED, vascular biology and cardiovascular events.

EPIDEMIOLOGY

Epidemiology underscores the etiologic role of diet and associated nutrient intake in hypertension. The transition from the Paleolithic diet to our modern diet has produced an epidemic of nutritionally-related diseases (Table 1). Hypertension, atherosclerosis, coronary heart disease (CHD), MI, congestive heart failure (CHF), cerebrovascular accidents (CVA), renal disease, type 2 diabetes mellitus (T2DM), metabolic syndrome (MS) and obesity are some of these diseases^[1,2]. Table 1 contrasts intake of nutrients involved in BP regulation during the Paleolithic Era and modern time. Evolution from a pre-agricultural, hunter-gatherer milieu to an agricultural, refrigeration society has imposed an unnatural and unhealthy nutritional selection process. In sum, diet has changed more than our genetics can adapt.

The human genetic makeup is 99.9% that of our Paleolithic ancestors, yet our nutritional, vitamin and mineral intakes are vastly different^[3]. The macronutrient and micronutrient variations, oxidative stress from radical oxygen species (ROS) and radical nitrogen species (RNS) and inflammatory mediators such as cell adhesion molecules (CAMs), cytokines, signaling molecules and autoimmune vascular dysfunction of T cells and B cells, contribute to the higher incidence of hypertension and other cardiovascular diseases through complex nutrient-gene interactions, epigenetic and nutrient-caveolae interactions and nutrient reactions with pattern recognition receptors [toll like receptors (TLR) and nod like receptors] in the endothelium^[4-9] (Figure 4). Reduction in nitric oxide bioavailability, increase in angiotensin II and endothelin coupled with endothelial activation initiate the vascular and cardiac dysfunction and hypertension. Poor nutrition, coupled with obesity and a sedentary lifestyle have resulted in an exponential increase in nutritionally-related diseases. In particular, the high Na⁺/K⁺ ratio of modern diets has contributed to hypertension, CVA, CHD, MI, CHF and renal disease^[3,10] as have the relatively low intake of omega-3 PUFA, increase in omega-6 PUFA, saturated fat and trans fatty acids^[11].

PATHOPHYSIOLOGY

Vascular biology assumes a pivotal role in the initiation and perpetuation of hypertension and cardiovascular TOD^[1]. Oxidative stress (ROS and RNS), inflammation (increased expression of redox-sensitive pro-inflammatory genes, CAMs and recruitment migration

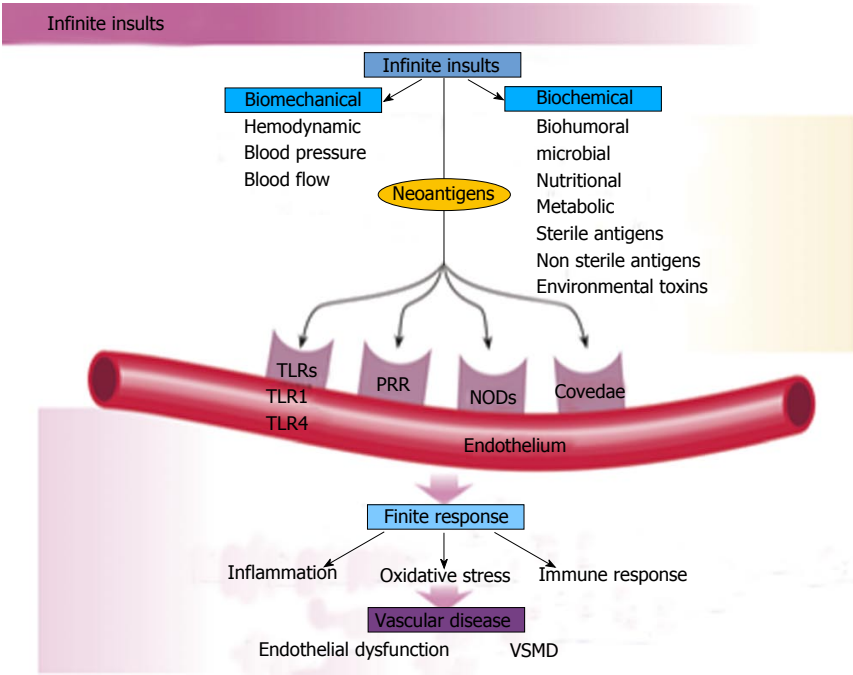


Figure 4 Infinite insults with three finite vascular responses. Biomechanical insults such as hypertension result in stimulation of pattern recognition receptors and caveolae that induce vascular inflammation, oxidative stress and immune dysfunction endothelial dysfunction and vascular and cardiac smooth muscle dysfunction. TLR: Toll like receptors.

Table 1 Dietary intake of nutrients involved in vascular biology: Comparing and contrasting the diet of paleolithic and contemporary humans

Nutrients and dietary characteristics	Paleolithic intake	Modern intake
Sodium	< 50 mmol/d (1.2 g)	175 mmol/d (4 g)
Potassium	> 10000 meq/d (256 g)	150 meq/d (6 g)
Sodium/potassium ratio	< 0.13/d	> 0.67/d
Protein	37%	20%
Carbohydrate	41%	40%-50%
Fat	22%	30%-40%
Polyunsaturated/saturated	1.4	0.4
Fat ratio		
Fiber	> 100 g/d	9 g/d

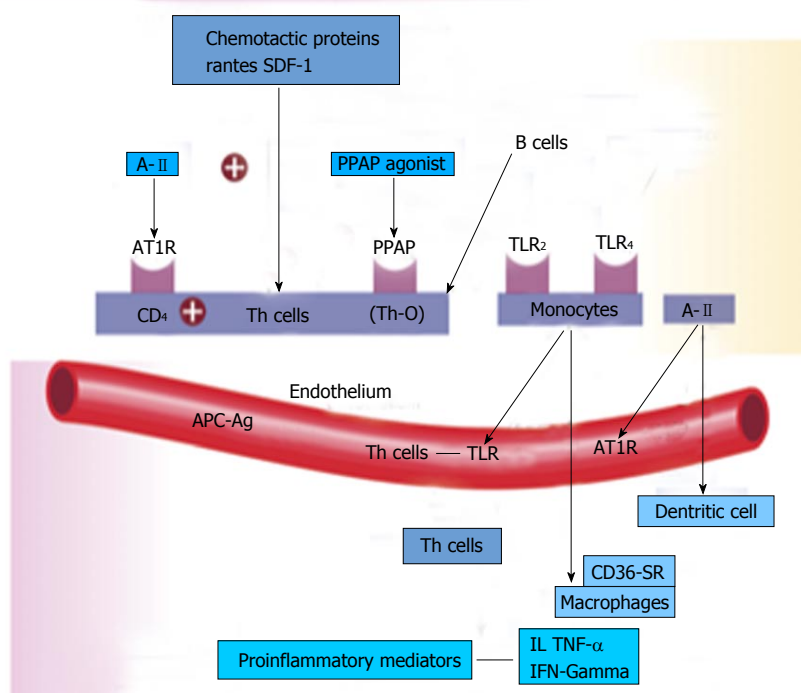
and infiltration of circulating cells) and autoimmune vascular dysfunction (T cells and B cells) are the primary pathophysiologic and functional mechanisms that induce vascular disease^[1,12-14] (Figure 5). All three of these are closely inter-related and establish a deadly combination that leads to ED, vascular smooth muscle and cardiac dysfunction, hypertension, vascular disease, atherosclerosis and CVD. Hypertension is not a disease but is the correct and chronically dysregulated response with an exaggerated outcome of the infinite insults to the blood vessel with subsequent environmental-genetic expression patterns and downstream disturbances in which the vascular system is the innocent bystander. This becomes a maladaptive vascular response that was initially intended to provide vascular defense to the endothelial insults (Figure 6)^[1,15-15]. Hypertension is a vasculopathy characterized by ED, structural remodeling, vascular inflammation, increased arterial stiffness, reduced distensibility and loss of elasticity^[13]. These insults are biomechanical (BP, pulse pressure, blood flow, oscillatory flow, turbulence, augmentation, pulse wave velocity and reflected

waves) and biohumoral or biochemical which includes all the non-mechanical causes such as metabolic, endocrine, nutritional, toxic, infectious and other etiologies^[1] (Figure 4). In addition to the very well established connections for endocrine and nutritional causes of hypertension, toxins and infections also increase BP^[16-20]. Various toxins such as polychlorinated biphenyls, mercury, lead, cadmium, arsenic and iron also increase BP and CVD^[16,17]. Numerous microbial organisms have been implicated in hypertension and CHD^[18-20]. All of these insults lead to impaired microvascular structure and function which manifests clinically as hypertension^[12-14]. The level of BP may not give an accurate indication of the microvascular involvement and impairment in hypertension. Hypertensive patients have abnormal microvasculature in the form of inward eutrophic remodeling of the small resistance arteries leading to impaired vasodilatory capacity, increased vascular resistance, increased media to lumen ratio, decreased maximal organ perfusion and reduced flow reserve, especially in the heart with decreased coronary flow reserve^[12-14]. Significant functional then structural microvascular impairment occurs even before the BP begins to rise in normotensive offspring of hypertensive parents evidenced by ED, impaired vasodilation, forearm vascular resistance, diastolic dysfunction, increased left ventricular mass index, increased septal and posterior wall thickness and left ventricular hypertrophy^[12,15]. Thus, the cellular processes underlying the vascular perturbations constitute a vascular phenotype of hypertension that may be determined by early life programming and imprinting which is compounded by vascular aging^[12-14].

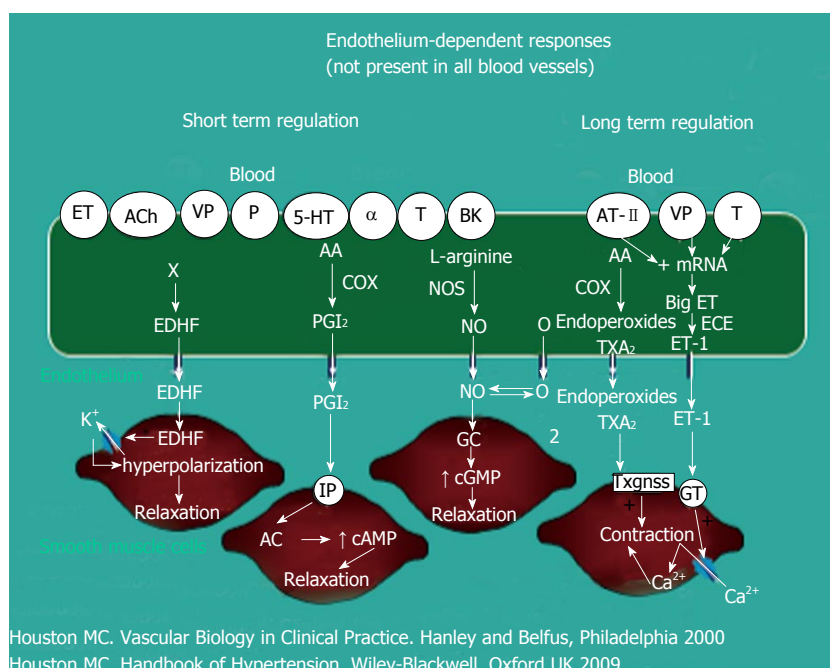
Oxidative stress

Oxidative stress, with an imbalance between ROS and RNS and the anti-oxidant defense mechanisms, contrib-

Autoimmune dysfunction in arteries

**Figure 5 Immune vascular dysfunction.**

Stimulation of the angiotensin receptor and toll like receptors and other and direct stimulation of T cells on the endothelium and vascular smooth muscle lead to immune dysfunction, inflammation and oxidative stress. TLR: Toll like receptors; IL: Interleukin; TNF- α : Tumor necrosis factor alpha.

**Figure 6 Stimulation of the AT1R increases production of superoxide anion which neutralizes nitric oxide and also forms additional downstream radical oxygen species and radical nitrogen species that increase vascular oxidative stress. AA: Arachidonic acid; NOS: Nitric oxide synthase.**

utes to the etiology of hypertension in animals^[10] and humans^[11,12]. Radical oxygen species and RNS are generated by multiple cellular sources, including nicotinamide adenine dinucleotide phosphate hydrazase (NADPH) oxidase, mitochondria, xanthine oxidase, uncoupled endothelium-derived nitric oxide (NO) synthase (U-eNOS), cyclooxygenase and lipo-oxygenase^[11]. Superoxide anion is the predominant ROS species produced by these tissues, which neutralizes NO and also leads to downstream production of other ROS (Figure 3). Hypertensive patients have impaired endogenous and exogenous anti-oxidant defense mechanisms^[21], an increased plasma oxidative

stress and an exaggerated oxidative stress response to various stimuli^[21,22]. Hypertensive subjects also have lower plasma ferric reducing ability of plasma, lower vitamin C levels and increased plasma 8-isoprostanes, which correlate with both systolic and diastolic BP. Various single-nucleotide polymorphisms (SNP's) in genes that codify for anti-oxidant enzymes are directly related to hypertension^[23]. These include NADPH oxidase, xanthine oxidase, superoxide dismutase 3 (SOD 3), catalase, glutathione peroxidase 1 (GPx 1) and thioredoxin. Antioxidant deficiency and excess free radical production have been implicated in human hypertension in numerous

Table 2 Oxidative stress induces endothelial dysfunction, vascular disease and hypertension. Host protective factors include enzymatic and non-enzymatic defenses influenced by diet and nutrients

The cytotoxic reactive oxygen species and the natural defense mechanisms			
Reactive oxygen species		Antioxidant defense mechanisms	
Free radicals		Enzymatic scavengers	
O ₂ • ⁻	Superoxide anion radical	SOD	Superoxide dismutase
OH•	Hydroxyl radical		2O ₂ • ⁻ + 2H ⁺ → H ₂ O ₂ + O ₂
ROO•	Lipid peroxide (peroxyl)	CAT	Catalase (peroxisomal-bound)
RO•	Alkoxy		2H ₂ O ₂ → O ₂ + H ₂ O
RS•	Thiyl	GTP	Glutathione peroxidase
NO•	Nitric oxide		2GSH + H ₂ O ₂ → GSSG + 2H ₂ O
NO ₂ •	Nitrogen dioxide		2GSH + ROOH → GSSG + ROH + 2H ₂ O
ONOO ⁻	Peroxynitrite		
CCl ₃ •	Trichloromethyl		
Non-radicals		Nonenzymatic scavengers	
H ₂ O ₂	Hydrogen peroxide	Vitamin A	
HOCl	Hypochlorous acid	Vitamin C (ascorbic acid)	
ONOO ⁻	Peroxynitrite	Vitamin E (α-tocopherol)	
¹ O ₂	Singlet oxygen	β-carotene	
The superscripted bold dot indicates an unpaired electron and the negative charge indicates a gained electron. GSH, reduced glutathione; GSSG, oxidized glutathione; R, lipid chain. Singlet oxygen is an unstable molecule due to the two electrons present in its outer orbit spinning in opposite directions.		Cysteine	
		Coenzyme Q	
		Uric acid	
		Flavonoids	
		Sulphydryl group	
		Thioether compounds	

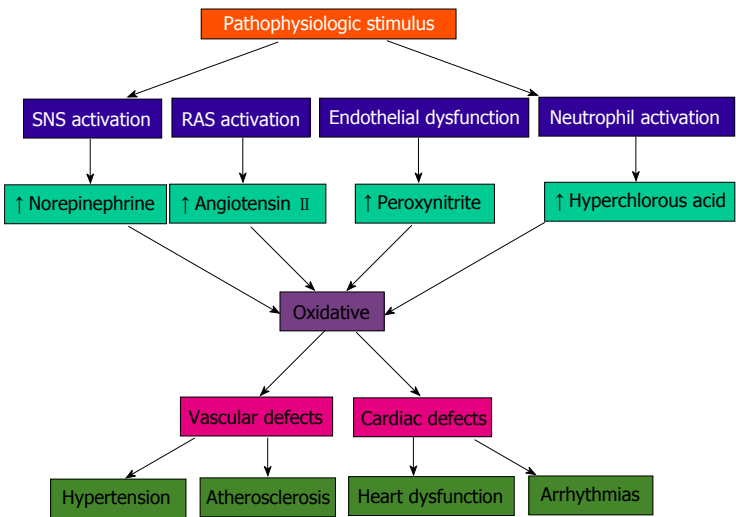


Figure 7 Neurohormonal and oxidative stress system interaction on cardiac and vascular muscle. SNS: Sympathetic nervous system; RAS: Renin angiotensin (aldosterone) system.

epidemiologic, observational and interventional studies (Table 2)^[21,22,24]. Radical oxygen species directly damage endothelial cells, degrade NO, influence eicosanoid metabolism, oxidize LDL, lipids, proteins, carbohydrates, DNA and organic molecules, increase catecholamines, damage the genetic machinery, influence gene expression and transcription factors^[1,21,22,25,26]. The inter-relations of neurohormonal systems, oxidative stress and cardiovascular disease are shown in Figures 6 and 7. The increased oxidative stress, inflammation and autoimmune vascular dysfunction in human hypertension results from a combination of increased generation of ROS and RNS, an exacerbated response to ROS and RNS and a decreased antioxidant reserve^[24-29]. Increased oxidative stress in the rostral ventrolateral medulla (RVLM) enhances glutamatergic excitatory inputs and attenuates GABA-ergic inhibitory inputs to the RVLM which contributes to increased sympathetic nervous system (SNS) activity from

the paraventricular nucleus^[30]. Activation of the AT1R in the RVLM increases NADPH oxidase and increases oxidative stress and superoxide anion, increases SNS outflow causing an imbalance of SNS/PNS activity with elevation of BP, increased heart rate and alterations in heart rate variability and heart rate recovery time, which can be blocked by AT1R blockers^[30,31].

Inflammation

The link between inflammation and hypertension has been suggested in both cross-sectional and longitudinal studies^[32]. Increases in high sensitivity C-reactive protein (HS-CRP) as well as other inflammatory cytokines such as interleukin-1B, (IL-1B), IL-6, tumor necrosis alpha (TNF-α) and chronic leukocytosis occur in hypertension and hypertensive- related TOD, such as increased carotid IMT^[33]. HS-CRP predicts future CV events^[32,33]. Elevated HS-CRP is both a risk marker and risk factor

for hypertension and CVD^[34,35]. Increases in HS-CRP of over 3 µg/mL may increase BP in just a few days that is directly proportional to the increase in HS-CRP^[34,35]. Nitric oxide and eNOS are inhibited by HS-CRP^[34,35]. The AT2R, which normally counterbalances AT1R, is down-regulated by HS-CRP^[34,35]. Angiotensin II (A-II) upregulates many of the cytokines, especially IL-6, CAMs and chemokines by activating nuclear factor Kappa B (NF-κB) leading to vasoconstriction. These events, along with the increases in oxidative stress and endothelin-1, elevate BP^[32].

Autoimmune dysfunction

Innate and adaptive immune responses are linked to hypertension and hypertension-induced CVD through at least three mechanisms: cytokine production, central nervous system stimulation and renal damage. This includes salt-sensitive hypertension with increased renal inflammation as a result of T cell imbalance, dysregulation of CD4⁺ and CD8⁺ lymphocytes and chronic leukocytosis with increased neutrophils and reduced lymphocytes^[36-38]. Leukocytosis, especially increased neutrophils and decreased lymphocyte count increase BP in Blacks by 6/2 mmHg in the highest *vs* the lowest tertile^[38]. Macrophages and various T-cell subtypes regulate BP, invade the arterial wall, activate TLRs and induce autoimmune vascular damage^[38,39]. Angiotensin II activates immune cells (T cells, macrophages and dendritic cells) and promotes cell infiltration into target organs^[39]. CD4⁺ T lymphocytes express AT1R and PPAR gamma receptors, and release TNF-α, interferon and interleukins within the vascular wall when activated^[39] (Figure 5). IL-17 produced by T cells may play a pivotal role in the genesis of hypertension caused by Angiotensin II^[39]. Hypertensive patients have significantly higher TLR 4 mRNA in monocytes compared to normal^[40]. Intensive reduction in BP to systolic BP (SBP) less than 130 mmHg *vs* SBP to only 140 mmHg lowers the TLR 4 more^[40]. A-II activates the TLR expression leading to inflammation and activation of the innate immune system. When TLR 4 is activated there is downstream macrophage activation, migration, increase metalloproteinase 9, vascular remodeling, collagen accumulation in the artery, LVH and cardiac fibrosis^[40]. The autonomic nervous system is critical in either increasing or decreasing immune dysfunction and inflammation^[41]. Efferent cholinergic anti-inflammatory pathways *via* the vagal nerve innervate the spleen, nicotine acetylcholine receptor subunits and cytokine producing immune cells to influence vasoconstriction and BP^[41]. Local CNS inflammation or ischemia may mediate vascular inflammation and hypertension^[39].

Aldosterone is associated with increased adaptive immunity and autoimmune responses with CD4⁺ T cell activation and Th 17 polarization with increased IL 17, TGF-β and TNF-α which modulate over 30 inflammatory genes^[42,43]. Increased serum aldosterone is an independent risk factor for CVD and CHD through non-hemodynamic effects as well as through increased

BP^[42,43]. Blockade of mineralocorticoid receptors in the heart, brain, blood vessels and immune cells reduces CV risk even with the persistence of hypertension^[42,43].

TREATMENT

Many of the natural compounds in food, certain nutraceutical supplements, vitamins, antioxidants or minerals function in a similar fashion to a specific class of antihypertensive drugs. Although the potency of these natural compounds may be less than the antihypertensive drug, when used in combination with other nutrients and nutraceutical supplements, the antihypertensive effect is additive or synergistic. Table 3 summarizes these natural compounds into the major antihypertensive drug classes such as diuretics, beta blockers, central alpha agonists, direct vasodilators, calcium channel blockers (CCB's), angiotensin converting enzyme inhibitors (ACEI's), angiotensin receptor blockers (ARB's) and direct renin inhibitors (DRI).

Dietary Approaches to Stop Hypertension diets

The Dietary Approaches to Stop Hypertension (DASH) I and II diets conclusively demonstrated significant reductions in BP in borderline and stage I hypertensive patients^[44,45]. In DASH I untreated hypertensive subjects with SBP < 160 mmHg and DBP 80-95 mmHg were placed on one of three diets for 4 wk, control diet, fruit and vegetable diet (F + V) and combined diet that added F + V and low fat dairy^[44]. DASH II added progressive sodium restriction in each group^[45]. The control diet consisted of sodium at 3 g/d, potassium, magnesium and calcium at 25% of the US average, macronutrients at US average of 4 servings per day, a sodium/potassium ratio of 1.7 and fiber at 9 g/d. The F + V diet increased the potassium, magnesium and calcium to 75%, macronutrients to greater than the US average, a sodium/potassium ratio of 0.7, 31 g of fiber and 8.5 servings of fruits and vegetables per day. The combined diet was similar to the F + V diet but added low fat dairy. At 2 wk the BP was decreased by 10.7/5.2 mmHg in the hypertensive patients in DASH I and 11.5/6.8 mmHg in the hypertensive patients in DASH II. These reductions persisted as long as the patients were on the diet. The DASH diet increases plasma renin activity (PRA) and serum aldosterone levels in response to the BP reductions^[46,47]. The mean increase in PRA was 37 ng/mL per day^[47]. There was an associated of response with the G46A polymorphism of beta 2 adrenergic receptor. The A allele of G46A had a greater BP reduction and blunted PRA and aldosterone. The arachidonic acid (AA) genotype had the best response and the GG genotype had no response. Adding an ARB, ACEI or DRI improved BP response to the DASH diet in the GG group due to blockade of the increase in PRA. A low sodium DASH diet decreases oxidative stress (urine F2-isoprostanes), improves vascular function (augmentation index) and lowers BP in salt sensitive subjects^[48]. In addition, plasma nitrite increased and pulse wave velocity

Table 3 Natural antihypertensive compounds categorized by antihypertensive class

Antihypertensive therapeutic class (alphabetical listing)	Foods and ingredients listed by therapeutic class	Nutrients and other supplements listed by therapeutic class
Angiotensin converting enzyme inhibitors	Egg yolk Fish (specific): bonito, dried salted fish, fish sauce sardine muscle/protein tuna garlic gelatin hawthorne berry Milk products (specific): casein sour milk whey (hydrolyzed) sake sea vegetables (kelp) sea weed (wakame) wheat germ (hydrolyzed) zein (corn protein)	Melatonin omega-3 fatty acids pomegranate pycnogenol zinc
Angiotensin receptor blockers	Celery fiber garlic MUFA	Coenzyme Q10 gamma linolenic acid N-acetyl cysteine oleic acid resveratrol potassium taurine vitamin C vitamin B6 (pyridoxine)
Beta blockers	Hawthorne berry	
Calcium channel blockers	Celery garlic hawthorn berry MUFA	Alpha lipoic acid calcium magnesium N-acetyl cysteine oleic acid omega-3 fatty acids: eicosapentaenoic acid docosahexaenoic acid taurine vitamin B6 vitamin C vitamin E
Central alpha agonists (reduce sympathetic nervous system activity)	Celery fiber garlic protein	Coenzyme Q 10 gamma linolenic acid potassium restriction of sodium taurine vitamin C vitamin B6 zinc Vitamin D
Direct renin inhibitors		
Direct vasodilators	Celery cooking oils with monounsaturated fats fiber garlic MUFA soy	Alpha linolenic acid arginine calcium flavonoids magnesium Omega-3 fatty acids potassium taurine vitamin C vitamin E
Diuretics	Celery hawthorn berry protein	Calcium coenzyme Q 10 fiber gamma linolenic acid l-carnitine magnesium potassium taurine vitamin B6 vitamin C Vitamin E: high gamma/delta tocopherols and tocotrienols.

MUFA: Monounsaturated fatty acids.

decreased at week two on the DASH diet^[49].**Sodium (Na⁺) reduction**

The average sodium intake in the US is 5000 mg/d with some areas of the country consuming 15000-20000 mg/d^[50]. However, the minimal requirement for sodium

is probably about 500 mg/d^[50]. Epidemiologic, observational and controlled clinical trials demonstrate that an increased sodium intake is associated with higher BP as well as increased risk for CVD, CVA, LVH, CHD, MI, renal insufficiency, proteinuria and over activity of the SNS^[1,50]. A reduction in sodium intake in hypertensive patients, especially the salt sensitive patients, will significantly lower BP by 4-6/2-3 mmHg that is proportional to the degree of sodium restriction and may prevent or delay hypertension in high risk patients and reduce future CV events^[51-53].

Salt sensitivity ($\geq 10\%$ increase in MAP with salt loading) occurs in about 51% of hypertensive patients and is a key factor in determining the cardiovascular, cerebrovascular, renal and BP responses to dietary salt intake^[54]. Cardiovascular events are more common in the salt sensitive patients than in salt resistant ones, independent of BP^[55]. An increased sodium intake has a direct positive correlation with BP and the risk of CVA and CHD^[56]. The risk is independent of BP for CVA with a relative risk of 1.04 to 1.25 from the lowest to the highest quartile^[56]. In addition, patients will convert to a nondipping BP pattern with increases in nocturnal BP as the sodium intake increases^[56].

Increased sodium intake has a direct adverse effect on endothelial cells^[57-61]. Sodium promotes cutaneous lymphangiogenesis, increases endothelial cell stiffness, reduces size, surface area, volume, cytoskeleton, deformability and pliability, reduces eNOS and NO production, increases asymmetric dimethyl arginine (ADMA), oxidative stress and TGF- β . All of these abnormal vascular responses are increased in the presence of aldosterone^[57-61]. These changes occur independent of BP and may be partially counteract by dietary potassium^[57-61]. The endothelial cells act as vascular salt sensors^[62]. Endothelial cells are targets for aldosterone which activate epithelial sodium channels (ENaCs) and have a negative effects on release of NO and on endothelial function. The mechanical stiffness of the cell plasma membrane and the submembranous actin network (endothelial glycocalyx) ("shell") serve as a "firewall" to protect the endothelial cells and are regulated by serum sodium, potassium and aldosterone within the physiologic range^[62]. Changes in shear-stress-dependent activity of the endothelial NO synthase located in the caveolae regulate the viscosity in this "shell"^[62]. High plasma sodium gelates the shell of the endothelial cell, whereas the shell is fluidized by high potassium. These communications between extracellular ions and intracellular enzymes occur at the plasma membrane barrier, whereas 90% of the total cell mass remains uninvolved in these changes. Blockade of the ENaC with spironolactone (100%) or amiloride (84%) minimizes or stop many of these vascular endothelial responses and increase NO^[58,63]. Nitric oxide release follows endothelial nanomechanics and not vice versa and membrane depolarization decreases vascular endothelial cell stiffness which improves flow mediated nitric-oxide dependent vasodilation^[64,65]. In the presence of vascular inflammation and increased HS-CRP, the effects of aldosterone on the

ENaC is enhanced further increasing vascular stiffness and BP^[66]. High sodium intake also abolishes the AT2R-mediated vasodilation immediately with complete abolition of endothelial vasodilation (EDV) within 30 d^[67]. Thus, it has become clear that increased dietary sodium has adverse effects on the vascular system, BP and CVD by altering the endothelial glycocalyx, which is a negatively charged biopolymer that lines the blood vessels and serves as a protective barrier against sodium overload, increased sodium permeability and sodium-induced TOD^[68]. Certain SNP's of salt inducible kinase I which alter Na⁺/K⁺ ATPase, determine sodium induced hypertension and LVH^[69].

The sodium intake per day in hypertensive patients should be between 1500 to 2000 mg. Sodium restriction improves BP reduction in those on patients that are on pharmacologic treatment and the decrease in BP is additive with restriction of refined carbohydrates^[70,71]. Reducing dietary sodium intake may reduce damage to the brain, heart, kidney and vasculature through mechanisms dependent on the small BP reduction as well as those independent of the decreased BP^[72-75].

A balance of sodium with other nutrients, especially potassium, magnesium and calcium is important, not only in reducing and controlling BP, but also in decreasing cardiovascular and cerebrovascular events^[3,72,73]. An increase in the sodium to potassium ratio is associated with significantly increased risk of CVD and all-cause mortality^[72]. The Yanomamo Indians consume and excrete only 1 meq of sodium in 24 h and consume and excrete 152 meq of potassium in 24 h^[73]. The Na⁺ to K⁺ ratio is 1/152 and is associated with elevated PRA, but BP does not increase with age. At age 50 the average BP in the Yanomamo is 100-108/64-69 mmHg^[73].

Potassium

The average U.S. dietary intake of potassium (K⁺) is 45 mmol/d with a potassium to sodium (K⁺/Na⁺) ratio of less than 1:2^[10,74]. The recommended intake of K⁺ is 4700 mg/d (120 mmol) with a K⁺/Na⁺ ratio of about 4-5 to 1^[10,74]. Numerous epidemiologic, observational and clinical trials have demonstrated a significant reduction in BP with increased dietary K⁺ intake in both normotensive and hypertensive patients^[10,74,76]. The average BP reduction with a K⁺ supplementation of 60 to 120 mmol/d is 4.4/2.5 mmHg in hypertensive patients but may be as much as 8/4.1 mmHg with 120 mmol/d (4700 mg)^[10,74,76,77]. In hypertensive patients, the linear dose-response relationship is 1.0 mmHg reduction in SBP and 0.52 mmHg reduction in diastolic BP per 0.6 g/d increase in dietary potassium intake that is independent of baseline dietary potassium ingestion^[10]. The response depends on race (black > white), sodium, magnesium and calcium intake^[10]. Those on a higher sodium intake have a greater reduction in BP with potassium^[10]. Alteration of the K⁺/Na⁺ ratio to a higher level is important for both anti-hypertensive as well as cardiovascular and cerebrovascular effects^[10,77]. High potassium intake reduces the incidence of cardiovascular (CHD, MI) and CVA independent of

the BP reduction^[10,74,76,77]. There are also reductions in CHF, LVH, diabetes mellitus and cardiac arrhythmias^[10]. If the serum potassium is less than 4.0 meq/dL, there is an increased risk of CVD mortality, ventricular tachycardia, ventricular fibrillation and CHF^[10]. Red blood cell potassium is a better indication of total body stores and CVD risk than is serum potassium^[10]. Gu *et al*^[77] found that potassium supplementation at 60 mmol of KCl per day for 12 wk significantly reduced SBP -5.0 mmHg (range -2.13 to -7.88 mmHg) ($P < 0.001$) in 150 Chinese men and women aged 35 to 64 years.

Potassium increases natriuresis, modulates baroreflex sensitivity, vasodilates, decreases the sensitivity to catecholamines and Angiotensin II, increases sodium potassium ATPase and DNA synthesis in the vascular smooth muscle cells and decreases SNS activity in cells with improved vascular function^[10]. In addition, potassium increases bradykinin and urinary kallikrein, decreases NADPH oxidase, which lowers oxidative stress and inflammation, improves insulin sensitivity, decreases ADMA, reduces intracellular sodium and lowers production of TGF- β ^[10].

Each 1000 mg increase in potassium intake per day reduces all cause mortality by approximately 20%. Potassium intake of 4.7 g/d is estimated to decrease CVA by 8% to 15% and MI by 6%-11%^[10]. Numerous SNP's such as nuclear receptor subfamily 3 group C, angiotensin II type receptor and hydroxysteroid 11 beta dehydrogenase (HSD11B1 and B2) determine an individual's response to dietary potassium intake^[78]. Each 1000 mg decrease in sodium intake per day will decrease all cause mortality by 20%^[10,73]. A recent analysis suggested a dose related response to CVA with urinary potassium excretion^[79]. There was a RRR of CVA of 23% at 1.5-1.99 g, 27% at 2.0-2.49 g, 29% at 2.5-3 g and 32% over 3 g/d of potassium urinary excretion^[79]. The recommended daily dietary intake for patients with hypertension is 4.7 to 5.0 g of potassium and less than 1500 mg of sodium^[10]. Potassium in food or from supplementation should be reduced or used with caution in those patients with renal impairment or those on medications that increase renal potassium retention such as ACEI, ARB, DRI and serum aldosterone receptor antagonists^[10].

Magnesium

A high dietary intake of magnesium of at least 500-1000 mg/d reduces BP in most of the reported epidemiologic, observational and clinical trials, but the results are less consistent than those seen with Na⁺ and K⁺^[74,80]. In most epidemiologic studies, there is an inverse relationship between dietary magnesium intake and BP^[74,80,81]. A study of 60 essential hypertensive subjects given magnesium supplements showed a significant reduction in BP over an eight week period documented by 24 h ambulatory BP, home and office blood BP^[74,80,81]. The maximum reduction in clinical trials has been 5.6/2.8 mmHg but some studies have shown no change in BP^[82]. The combination of high potassium and low sodium intake with increased magnesium intake had additive anti-hypertensive ef-

fects^[82]. Magnesium also increases the effectiveness of all anti-hypertensive drug classes^[82].

Magnesium competes with Na^+ for binding sites on vascular smooth muscle and acts as a direct vasodilator, like a CCB. Magnesium increases prostaglandin E (PGE), regulates intracellular calcium, sodium, potassium and pH, increases nitric oxide, improves endothelial function, reduces oxLDL, reduces HS-CRP, TBxA2, A-II, and nor-epinephrine. Magnesium also improves insulin resistance, glucose and MS, binds in a necessary-cooperative manner with potassium, inducing EDV and BP reduction, reduces CVD and cardiac arrhythmias, decreases carotid IMT, lowers cholesterol, lowers cytokine production, inhibits nuclear factor Kb, reduces oxidative stress and inhibits platelet aggregation to reduce thrombosis^[74,80-86].

Magnesium is an essential co-factor for the delta-6-desaturase enzyme that is the rate-limiting step for conversion of linoleic acid (LA) to gamma linolenic acid (GLA)^[74,80,81,83-85] needed for synthesis of the vasodilator and platelet inhibitor PGE₁. Altered TRPM7 channels, which are the transporter for magnesium occur in many hypertensive patients^[83].

A meta-analysis of 241378 patients with 6477 strokes showed an inverse relationship of dietary magnesium to the incidence of ischemic stroke^[84]. For each 100 mg of dietary magnesium intake, ischemic stroke was decreased by 8%. The proposed mechanism include inhibition of ischemia induced glutamate release, NMDA receptor blockade, CCB actions, mitochondrial calcium buffering, decrease in ATP depletion and vasodilation of the cerebral arteries^[84]. A meta-analysis showed reductions in BP of 3-4/2-3 mmHg in 22 trials of 1173 patients^[87].

Intracellular level of magnesium (RBC) is more indicative of total body stores and should be measured in conjunction with serum and urinary magnesium^[83]. Magnesium may be supplemented in doses of 500 to 1000 mg/d. Magnesium formulations chelated to an amino acid may improve absorption and decrease the incidence of diarrhea^[82]. Adding taurine at 1000 to 2000 mg/d will enhance the anti-hypertensive effects of magnesium^[82]. Magnesium supplements should be avoided or used with caution in patients with known renal insufficiency or in those taking medications that induce magnesium retention^[82].

Calcium

Population studies show a link between hypertension and calcium^[88], but clinical trials that administered calcium supplements to patients have shown inconsistent effects on BP^[88]. The heterogeneous responses to calcium supplementation have been explained by Resnick^[89]. This is the "ionic hypothesis"^[89] of hypertension, cardiovascular disease and associated metabolic, functional and structural disorders. Calcium supplementation is not recommended at this time as an effective means to reduce BP.

Zinc

Low serum zinc levels in observational studies correlate with hypertension as well as CHD, type II DM, hyperlipidemia, elevated lipoprotein a [Lp(a)], increased 2 h post-

prandial plasma insulin levels and insulin resistance^[90,91]. Zinc is transported into cardiac and vascular muscle and other tissues by metallothionein^[92]. Genetic deficiencies of metallothionein with intramuscular zinc deficiencies may lead to increased oxidative stress, mitochondrial dysfunction, cardiomyocyte dysfunction and apoptosis with subsequent myocardial fibrosis, abnormal cardiac remodeling, heart disease, heart failure, or hypertension^[92]. Intracellular calcium increases oxidative stress which is reduced by zinc^[92].

Bergomi *et al*^[93] evaluated Zinc (Zn^{++}) status in 60 hypertensive subjects compared to 60 normotensive control subjects. An inverse correlation of BP and serum Zn^{++} was observed. The BP was also inversely correlated to a Zn^{++} dependent enzyme-lysyl oxidase activity. Zn^{++} inhibits gene expression and transcription through NF- κ B and activated protein-1 and is an important cofactor for SOD^[90,92]. These effects plus those on insulin resistance, membrane ion exchange, RAAS and SNS effects may account for Zn^{++} antihypertensive effects^[90,92]. Zinc intake should be 50 mg/d^[1].

Protein

Observational and epidemiologic studies demonstrate a consistent association between a high protein intake and a reduction in BP and incident BP^[94,95]. The protein source is an important factor in the BP effect; animal protein being less effective than non-animal or plant protein, especially almonds^[94-97]. In the Inter-Salt Study of over 10000 subjects, those with a dietary protein intake 30% above the mean had a lower BP by 3.0/2.5 mmHg compared to those that were 30% below the mean (81 vs 44 g/d)^[94]. However, lean or wild animal protein with less saturated fat and more essential omega-3 fatty acids may reduce BP, lipids and CHD risk^[94,97]. A meta-analysis confirmed these findings and also suggested that hypertensive patients and the elderly have the greatest BP reduction with protein intake^[95]. Another meta-analysis of 40 trials with 3277 patients found reductions in BP of 1.76/1.15 mmHg compared to carbohydrate intake ($P < 0.001$)^[98]. Both vegetable and animal protein significantly and equally reduced BP at 2.27/1.26 mmHg and 2.54/0.95 mmHg respectively^[98]. Increased dietary protein intake is inversely associated with risk for stroke in women with hypertension^[99]. A randomized cross-over study in 352 adults with pre-hypertension and stage I hypertension found a significant reduction in SBP of 2.0 mmHg with soy protein and 2.3 mmHg with milk protein compared to a high glycemic index diet over each of the 8 wk treatment periods^[100]. There was a non-significant reduction in DBP. Another RDB parallel study over 4 wk of 94 subjects with prehypertension and stage I hypertension found significant reductions on office BP of 4.9/2.7 mmHg in those given a combination of 25% protein intake vs the control group given 15% protein in an isocaloric manner^[101]. The protein consisted of 20% pea, 20% soy, 30% egg and 30% milk-protein isolate^[101]. The daily recommended intake of protein from all sources is 1.0 to 1.5 g/kg body weight, varying with exercise level, age,

renal function and other factors^[1,70,71].

Fermented milk supplemented with whey protein concentrate significantly reduces BP in human studies^[102-106]. Administration of 20 g/d of hydrolyzed whey protein supplement rich in bioactive peptides significantly reduced BP over 6 wk by 8.0 ± 3.2 mmHg in SBP and 5.5 ± 2.1 mm in diastolic BP^[103]. Milk peptides which contain both caseins and whey proteins are a rich source of ACEI peptides. Val-Pro-Pro and Ile-Pro-Pro given at 5 to 60 mg/d have variable reductions in BP with an average decrease in pooled studies of about 1.28-4.8/0.59-2.2 mmHg^[71,100,104-107]. However several recent meta-analysis did not show significant reductions in BP in humans^[106,108]. Powdered fermented milk with *Lactobacillus helveticus* given at 12 g/d significantly lowered BP by 11.2/6.5 mmHg in 4 wk in one study^[104]. Milk peptides are beneficial in treating MS^[109]. A dose response study showed insignificant reductions in BP^[110]. The clinical response is attributed to fermented milk's active peptides which inhibit ACE.

Pins *et al*^[111] administered 20 g of hydrolyzed whey protein to 56 hypertensive subjects and noted a BP reduction of 11/7 mmHg compared to controls at one week that was sustained throughout the study. Whey protein is effective in improving lipids, insulin resistance, glucose, arterial stiffness and BP^[112]. These data indicate that the whey protein must be hydrolyzed in order to exhibit an antihypertensive effect, and the maximum BP response is dose dependent.

Bovine casein-derived peptides and whey protein-derived peptides exhibit ACEI activity^[102-111]. These components include B-caseins, B-Ig fractions, B2-microglobulin and serum albumin^[102-104,111]. The enzymatic hydrolysis of whey protein isolates releases ACEI peptides.

Marine collagen peptides (MCPs) from deep sea fish have anti-hypertensive activity^[113-115]. A double-blind placebo controlled trial in 100 hypertensive subjects with diabetes who received MCPs twice a day for 3 mo had significant reductions in DBP and mean arterial pressure^[113]. Bonito protein (*Sarda Orientalis*), from the tuna and mackerel family has natural ACEI inhibitory peptides and reduces BP 10.2/7 mmHg at 1.5 g/d^[114,116].

Sardine muscle protein, which contains Valyl-Tyrosine (VAL-TYR), significantly lowers BP in hypertensive subjects^[117]. Kawasaki *et al*^[117] treated 29 hypertensive subjects with 3 mg of VAL-TYR sardine muscle concentrated extract for four wk and lowered BP 9.7/5.3 mmHg ($P < 0.05$). Levels of A-I increased as serum A-II and aldosterone decreased indicating that VAL-TYR is a natural ACEI. A similar study with a vegetable drink with sardine protein hydrolysates significantly lowered BP by 8/5 mmHg in 13 wk^[118].

Soy protein lowers BP in hypertensive patients in most studies^[100,119-127]. Soy protein intake was significantly and inversely associated with both SBP and DBP in 45694 Chinese women consuming 25 g/d or more of soy protein over 3 years and the association increased with age^[119]. The SBP reduction was 1.9 to 4.9 mm lower and the DBP 0.9 to 2.2 mmHg lower^[119]. However, randomized clinical trials and meta-analysis have shown mixed

results on BP with no change in BP to reductions of 7% to 10 % for SBP and DBP^[121-125]. The recent meta-analysis of 27 trials found a significant reduction in BP of 2.21/1.44 mmHg^[120]. Some studies suggest improvement in endothelial function, improved arterial compliance, reduction in HS-CRP and inflammation, ACEI activity, reduction in sympathetic tone, diuretic action and reduction in both oxidative stress and aldosterone levels^[125-127]. Fermented soy at about 25 g/d is recommended.

In addition to ACEI effects, protein intake may also alter catecholamine responses and induce a natriuretic effect^[117,118]. Low protein intake coupled with low omega 3 fatty acid intake may contribute to hypertension in animal models^[128]. The optimal protein intake, depending on level of activity, renal function, stress and other factors, is about 1.0 to 1.5 g/kg per day^[1].

Amino acids and related compounds

L-arginine: L-arginine and endogenous methylarginines are the primary precursors for the production of NO, which has numerous beneficial cardiovascular effects, mediated through conversion of L-arginine to NO by eNOS. Patients with hypertension, hyperlipidemia, diabetes mellitus and atherosclerosis have increased levels of HSCRP and inflammation, increased microalbumin, low levels of apelin (stimulates NO in the endothelium), increased levels of arginase (breaks down arginine) and elevated serum levels of ADMA, which inactivates NO^[129-133].

Under normal physiological conditions, intracellular arginine levels far exceed the Km [Michaelis Menton constant(MMC)] of eNOS which is less than 5 μmol ^[134]. However, endogenous NO formation is dependent on extracellular arginine concentration^[134]. The intracellular concentrations of L-arginine are 0.1-3.8 mmol/L in endothelial cells while the plasma concentration of arginine is 80-120 μmol /L which is about 20-25 times greater than the MMC^[135,136]. Despite this, cellular NO formation depends on exogenous L-arginine and this is the arginine paradox. Renal arginine regulates BP and blocks the formation of endothelin, reduces renal sodium reabsorption and is a potent antioxidant^[134]. The NO production in endothelial cells is closely coupled to cellular arginine uptake indicating that arginine transport mechanisms play a major role in the regulation of NO-dependent function. Exogenous arginine can increase renal vascular and tubular NO bioavailability and influence renal perfusion, function and BP^[132]. Molecular eNOS uncoupling may occur in the absence of tetrahydrobiopterin which stabilizes eNOS, which leads to production of ROS^[135].

Human studies in hypertensive and normotensive subjects of parenteral and oral administrations of L-arginine demonstrate an antihypertensive effect as well as improvement in coronary artery blood flow and peripheral blood flow in PAD^[129,136-140]. The BP decreased by 6.2/6.8 mmHg on 10 g/d of L-arginine when provided as a supplement or through natural foods to a group of hypertensive subjects^[136]. Arginine produces a statistically and biologically significant decrease in BP and improved met-

abolic effect in normotensive and hypertensive humans that is similar in magnitude to that seen in the DASH-I diet^[136]. Arginine given at 4 g/d also significantly lowered BP in women with gestational hypertension without proteinuria, reduced the need for anti-hypertensive therapy, decreased maternal and neonatal complications and prolonged the pregnancy^[137,138]. The combination of arginine (1200 mg/d) and N-acetyl cysteine (NAC) (600 mg bid) administered over 6 mo to hypertensive patients with type 2 diabetes, lowered SBP and DBP ($P < 0.05$), increased HDL-C, decreased LDL-C and oxLDL, reduced HSCR, ICAM, VCAM, PAI-1, fibrinogen and IMT^[139]. A study of 54 hypertensive subjects given arginine 4 g three times per day for four weeks had significant reductions in 24 h ABM^[140]. A meta-analysis of 11 trials with 383 subjects administered arginine 4-24 g/d found average reduction in BP of 5.39/2.66 mmHg ($P < 0.001$) in 4 wk^[141]. Although these doses of L-arginine appear to be safe, no long term studies in humans have been published at this time and there are concerns of a pro-oxidative effect or even an increase in mortality in patients who may have severely dysfunctional endothelium, advanced atherosclerosis, CHD, ACS or MI^[142]. In addition to the arginine-NO path, there exists a nitrate/nitrite pathway that is related to dietary nitrates from vegetables, beetroot juice and the DASH diet that are converted to nitrites by symbiotic, salivary, GI and oral bacteria^[143]. Administration of beetroot juice or extract at 500 mg/d will increase nitrites and lower BP, improve endothelial function, increase cerebral, coronary and peripheral blood flow^[143].

L-carnitine and acetyl-L-carnitine: L-carnitine is a nitrogenous constituent of muscle primarily involved in the oxidation of fatty acids in mammals. Animal studies indicate that carnitine has both systemic anti-hypertensive effects as well as anti-oxidant effects in the heart by up-regulation of eNOS and PPAR gamma, inhibition of RAAS, modulation of NF- κ B and down regulation of NOX2, NOX4, TGF- β and CTGF that reduces cardiac fibrosis^[144,145]. Endothelial function, NO and oxidative defense are improved while oxidative stress and BP are reduced^[144-147].

Human studies on the effects of L-carnitine and acetyl-L-carnitine are limited, with minimal to no change in BP^[148-153]. In patients with MS, acetyl-L-carnitine at one gram bid over 8 wk, improved dysglycemia and reduced SBP by 7-9 mmHg, but diastolic BP was significantly decreased only in those with higher glucose^[151]. Low carnitine levels are associated with a nondipping BP pattern in Type 2 DM^[153]. Carnitine has antioxidant and anti-inflammatory effects and may be useful in the treatment of essential hypertension, type II DM with hypertension, hyperlipidemia, cardiac arrhythmias, CHF and cardiac ischemic syndromes^[1,149,150,153]. Doses of 2-3 g twice per day are recommended.

Taurine: Taurine is a sulfonic beta-amino acid that is considered a conditionally-essential amino acid, which is not utilized in protein synthesis, but is found free or

in simple peptides with its highest concentration in the brain, retina and myocardium^[154]. In cardiomyocytes, it represents about 50% of the free amino acids and has a role of an osmoregulator, inotropic factor and anti-hypertensive agent^[155].

Human studies have noted that essential hypertensive subjects have reduced urinary taurine as well as other sulfur amino acids^[1,154,155]. Taurine lowers BP, SVR and HR, decreases arrhythmias, CHF symptoms and SNS activity, increases urinary sodium and water excretion, increases atrial natriuretic factor, improves insulin resistance, increases NO and improves endothelial function. Taurine also decreases A-II, PRA, aldosterone, SNS activity, plasma norepinephrine, plasma and urinary epinephrine, lowers homocysteine, improves insulin sensitivity, kinins and acetyl choline responsiveness, decreases intracellular calcium and sodium, lowers response to beta receptors and has antioxidant, anti-atherosclerotic and anti-inflammatory activities, decreases IMT and arterial stiffness and may protect from risk of CHD^[1,154-160]. A lower urinary taurine is associated with increased risk of hypertension and CVD^[160,161]. A study of 31 Japanese males with essential hypertension placed on an exercise program for 10 wk showed a 26% increase in taurine levels and a 287% increase in cysteine levels. The BP reduction of 14.8/6.6 mmHg was proportional to increases in serum taurine and reductions in plasma norepinephrine^[162]. Fujita *et al*^[155] demonstrated a reduction in BP of 9/4.1 mmHg ($P < 0.05$) in 19 hypertension subjects given 6 g of taurine for 7 d. Taurine has numerous beneficial effects on the cardiovascular system and BP^[156]. The recommended dose of taurine is 2 to 3 g/d at which no adverse effects are noted, but higher doses up to 6 g/d may be needed to reduce BP significantly^[1,70,71,154-162].

Omega-3 fats

The omega-3 fatty acids found in cold water fish, fish oils, flax, flax seed, flax oil and nuts lower BP in observational, epidemiologic and in prospective clinical trials^[163-173]. The findings are strengthened by a dose-related response in hypertension as well as a relationship to the specific concomitant diseases associated with hypertension^[163-173].

Studies indicate that DHA at 2 g/d reduces BP and heart rate^[163,173]. The average reduction in BP is 8/5 mmHg and heart rate falls about 6 beats/min usually in about 6 wk^[1,70,71,91-175]. Fish oil at 4-9 g/d or combination of DHA and EPA at 3-5 g/d will also reduce BP^[1,168-173]. However, formation of EPA and ultimately DHA from ALA is decreased in the presence of high LA (the essential omega-6 fatty acid), saturated fats, trans fatty acids, alcohol, several nutrient deficiencies (magnesium, vitamin B6) and aging, all of which inhibit the desaturase enzymes^[163]. Eating cold water fish three times per week may be as effective as high dose fish oil in reducing BP in hypertensive patients, and the protein in the fish may also have antihypertensive effects^[1,163]. In patients with chronic kidney disease 4 g of omega 3 fatty acids reduced BP measured with 24 h ABM over 8 wk by 3.3/2.9 mmHg

compared to placebo ($P < 0.0001$)^[167].

The ideal ratio of omega-6 FA to omega-3 FA is between 1:1 to 1:4 with a polyunsaturated to saturated fat ratio greater than 1.5 to 2:0^[2]. Omega 3 fatty acids increase eNOS and nitric oxide, improve endothelial function, improve insulin sensitivity, reduce calcium influx, suppress ACE activity and improve parasympathetic tone^[1,163-171]. The omega-6 FA family includes LA, GLA, dihomo-GLA and AA which do not usually lower BP significantly, but may prevent increases in BP induced by saturated fats^[176]. GLA may block stress-induced hypertension by increasing PGE1 and PGI2, reducing aldosterone levels, reducing adrenal AT1R density and affinity^[175].

The omega-3 FA have a multitude of other cardiovascular consequences which modulates BP such as increases in eNOS and nitric oxide, improvement in ED, reduction in plasma nor-epinephrine and increase in paraSNS tone, suppression of ACE activity and improvement in insulin resistance^[176]. The recommended daily dose is 3000 to 5000 mg/d of combined DHA and EPA in a ratio of 3 parts EPA to 2 parts DHA and about 50% of this dose as GLA combined with gamma/delta tocopherol at 100 mg per gram of DHA and EPA to get the omega 3 index to 8% or higher to reduce BP and provide optimal cardioprotection^[177]. DHA is more effective than EPA for reducing BP and should be given at 2 g/d if administered alone^[163,173].

Omega-9 fats

Olive oil is rich in the omega-9 monounsaturated fat (MUFA) oleic acid, which has been associated with BP and lipid reduction in Mediterranean and other diets^[178-180]. Olive oil and MUFAs have shown consistent reductions in BP in most clinical studies in humans^[178-190]. In one study, the SBP fell 8 mmHg ($P \leq 0.05$) and the DBP fell 6 mmHg ($P \leq 0.01$) in both clinic and 24 h ambulatory BP monitoring in the MUFA treated subjects compared to the PUFA treated subjects^[178]. In addition, the need for antihypertensive medications was reduced by 48% in the MUFA group *vs* 4% in the omega-6 PUFA group ($P < 0.005$). Extra virgin olive oil (EVOO) was more effective than sunflower oil in lowering SBP in a group of 31 elderly hypertensive patients in a double blind randomized crossover study^[187]. The SBP was 136 mmHg in the EVOO treated subjects *vs* 150 mmHg in the sunflower treated group ($P < 0.01$). Olive oil also reduces BP in hypertensive diabetic subjects^[188]. It is the high oleic acid content in olive oil that reduces BP^[180]. In stage I hypertensive patients, oleuropein-olive leaf (*Olea Europaea*) extract 500 mg bid for 8 wk reduced BP 11.5/4.8 mmHg which was similar to captopril 25 mg bid^[189]. *Olea Europaea* L aqueous extract administered to 12 patients with hypertension at 400 mg qid for 3 mo significantly reduced BP ($P < 0.001$)^[181]. Olive oil intake in the EPIC study of 20343 subjects was inversely associated with both systolic and diastolic BP^[182]. In the SUN study of 6863 subjects, BP was inversely associated with olive oil consumption, but only in men^[183]. In a study of 40 hypertensive

monozygotic twins, olive leaf extract demonstrated a dose response reduction in BP at doses of 500 to 1000 mg/d in 8 wk compared to placebo^[184]. The low dose groups decreased BP 3/1 mmHg and the high dose 11/4 mmHg^[184]. A double blind, randomized, crossover dietary intervention study over 4 mo using polyphenol rich olive oil 30 mg/d decreased BP in the study group by 7.91/6.65 mmHg and improved endothelial function^[185]. The ADMA levels, oxLDL and HS-CRP were reduced in the olive oil group. Plasma nitrites and nitrates increased and hyperemic area after ischemia improved in the treated group. Olive oil inhibits the AT1R receptor, exerts L-type calcium channel antagonist effects and improves wave reflections and augmentation index^[191-193].

EVOO is also contains lipid-soluble phytonutrients such as polyphenols. Approximately 5 mg of phenols are found in 10 g of EVOO^[178,186]. About 4 tablespoons of EVOO is equal to 40 g of EVOO which is the amount required to get significant reductions in BP.

Fiber

The clinical trials with various types of fiber to reduce BP have been inconsistent^[194,195]. Soluble fiber, guar gum, guava, psyllium and oat bran may reduce BP and reduce the need for antihypertensive medications in hypertensive subjects, diabetic subjects and hypertensive-diabetic subjects^[1,70,71,194,195]. The average reduction in BP is about 7.5/5.5 mmHg on 40 to 50 g/d of a mixed fiber. There is improvement in insulin sensitivity, endothelial function, reduction in SNS activity and increase in renal sodium loss^[1,70,71,194].

Vitamin C

Vitamin C is a potent water-soluble electron-donor. At physiologic levels it is an antioxidant although at supra-physiologic doses such as those achieved with intravenous vitamin C it donates electrons to different enzymes which results in pro-oxidative effects. At physiologic doses vitamin C recycles vitamin E, improves ED and produces a diuresis^[196]. Dietary intake of vitamin C and plasma ascorbate concentration in humans is inversely correlated to SBP, DBP and heart rate^[196-210].

An evaluation of published clinical trials indicate that vitamin C dosing at 250 mg twice daily will significantly lower SBP 5-7 mmHg and diastolic BP 2-4 mmHg over 8 wk^[196-210]. Vitamin C will induce a sodium water diuresis, improve arterial compliance, improve endothelial function, increase nitric oxide and PGI2, decrease adrenal steroid production, improve sympathovagal balance, increase RBC Na/K ATPase, increase SOD, improve aortic elasticity and compliance, improve flow mediated vasodilation, decrease pulse wave velocity and augmentation index, increase cyclic GMP, activate potassium channels, reduce cytosolic calcium and reduce serum aldehydes^[208]. Vitamin C prevents ED induced by an oral glucose load. Vitamin C enhances the efficacy of amlodipine, decreases the binding affinity of the AT 1 receptor for angiotensin II by disrupting the ATR1 disulfide bridges and enhances the anti-hypertensive effects of medications in the elderly

with refractory hypertension^[1,70,71,200-205]. In elderly patients with refractory hypertension already on maximum pharmacologic therapy, 600 mg of vitamin C daily lowered the BP by 20/16 mmHg^[205]. The lower the initial ascorbate serum level, the better is the BP response. A serum level of 100 $\mu\text{mol/L}$ is recommended^[1,70,71]. The SBP and 24 ABM show the most significant reductions with chronic oral administration of Vitamin C^[200-205]. Block *et al*^[206] in an elegant depletion-repletion study of vitamin C demonstrated an inverse correlation of plasma ascorbate levels, SBP and DBP. In a meta-analysis of thirteen clinical trials with 284 patients, vitamin C at 500 mg/d over 6 wk reduced SBP 3.9 mmHg and DBP 2.1 mmHg^[207]. Hypertensive subjects were found to have significantly lower plasma ascorbate levels compared to normotensive subjects (40 $\mu\text{mol/L}$ vs 57 $\mu\text{mol/L}$ respectively)^[211], and plasma ascorbate is inversely correlated with BP even in healthy, normotensive individuals^[206].

Vitamin E

Most studies have not shown reductions in BP with most forms of tocopherols or tocotrienols^[1,70,71]. Patients with T2DM and controlled hypertension (130/76 mmHg) on prescription medications with an average BP of 136/76 mmHg were administered mixed tocopherols containing 60% gamma, 25% delta and 15% alpha tocopherols^[212]. The BP actually increased by 6.8/3.6 mmHg in the study patients ($P < 0.0001$) but was less compared to the increase with alpha tocopherol of 7/5.3 mmHg ($P < 0.0001$). This may be a reflection of drug interactions with tocopherols *via* cytochrome P 450 (3A4 and 4F2) and reduction in the serum levels of the pharmacologic treatments that were simultaneously being given^[212]. Gamma tocopherol may have natriuretic effects by inhibition of the 70pS potassium channel in the thick ascending limb of the loop of Henle and lower BP^[213]. Both alpha and gamma tocopherol improve insulin sensitivity and enhance adiponectin expression *via* PPAR gamma dependent processes, which have the potential to lower BP and serum glucose^[214]. If vitamin E has an antihypertensive effect, it is probably small and may be limited to untreated hypertensive patients or those with known vascular disease or other concomitant problems such as diabetes or hyperlipidemia.

Vitamin D

Vitamin D3 may have an independent and direct role in the regulation of BP and insulin metabolism^[215-225]. Vitamin D influences BP by its effects on calcium-phosphate metabolism, RAA system, immune system, control of endocrine glands and ED^[216]. If the Vitamin D level is below 30 ng/mL the circulating PRA levels are higher which increases angiotensin II, increases BP and blunts plasma renal blood flow^[221]. The lower the level of Vitamin D, the greater the risk of hypertension, with the lowest quartile of serum Vitamin D having a 52% incidence of hypertension and the highest quartile having a 20% incidence^[221]. Vitamin D3 markedly suppresses renin transcription by a VDR-mediated mechanism *via* the JGA ap-

paratus. Its role in electrolytes, volume and BP homeostasis indicates that Vitamin D3 is important in amelioration of hypertension. Vitamin D lower ADMA, suppresses pro-inflammatory cytokines such as TNF- α , increases nitric oxide, improves endothelial function and arterial elasticity, decreases vascular smooth muscle hypertrophy, regulates electrolytes and blood volume, increases insulin sensitivity, reduces free fatty acid concentration, regulates the expression of the natriuretic peptide receptor and lowers HS-CRP^[217-219,221].

The hypotensive effect of vitamin D was inversely related to the pretreatment serum levels of 1,25(OH) $_2$ D $_3$ and additive to antihypertensive medications. Pfeifer *et al*^[225] showed that short-term supplementation with vitamin D3 and calcium is more effective in reducing SBP than calcium alone. In a group of 148 women with low 25(OH) $_2$ D $_3$ levels, the administration of 1200 mg calcium plus 800 IU of vitamin D3 reduced SBP 9.3% more ($P < 0.02$) compared to 1200 mg of calcium alone. The HR fell 5.4% ($P = 0.02$), but DBP was not changed. The range in BP reduction was 3.6/3.1 to 13.1/7.2 mmHg. The reduction in BP is related to the pretreatment level of vitamin D3, the dose of vitamin D3 and serum level of vitamin D3, but BP is reduced only in hypertensive patients. Although vitamin D deficiency is associated with hypertension in observational studies, randomized clinical trials and their meta-analysis have yielded inconclusive results^[223]. In addition, vitamin D receptor gene polymorphisms may effect the risk of hypertension in men^[224]. A 25 hydroxyvitamin D level of 60 ng/mL is recommended.

Vitamin B6 (pyridoxine)

Low serum vitamin B6 (pyridoxine) levels are associated with hypertension in humans^[226]. One human study by Aybak *et al*^[227] proved that high dose vitamin B6 at 5 mg/kg per day for 4 wk significantly lowered BP by 14/10 mmHg. Pyridoxine (vitamin B6) is a cofactor in neurotransmitter and hormone synthesis in the central nervous system (norepinephrine, epinephrine, serotonin, GABA and kynurenine), increases cysteine synthesis to neutralize aldehydes, enhances the production of glutathione, blocks calcium channels, improves insulin resistance, decreases central sympathetic tone and reduces end organ responsiveness to glucocorticoids and mineralocorticoids^[1,70,71,228,229]. Vitamin B6 is reduced with chronic diuretic therapy and heme pyrollactams. Vitamin B6 thus has similar action to central alpha agonists, diuretics and CCB's. The recommended dose is 200 mg/d orally.

Flavonoids

Over 4000 naturally occurring flavonoids have been identified in such diverse substances as fruits, vegetables, red wine, tea, soy and licorice^[230]. Flavonoids (flavonols, flavones and isoflavones) are potent free radical scavengers that inhibit lipid peroxidation, prevent atherosclerosis, promote vascular relaxation and have antihypertensive properties^[230]. In addition, they reduce stroke and provide cardioprotective effects that reduce CHD morbidity and

mortality^[231].

Resveratrol is a potent antioxidant and antihypertensive found in the skin of red grapes and in red wine. Resveratrol administration to humans reduces augmentation index, improves arterial compliance and lowers central arterial pressure when administered as 250 mL of either regular or dealcoholized red wine^[232]. There was a significant reduction in the aortic augmentation index of 6.1% with the dealcoholized red wine and 10.5% with regular red wine. The central arterial pressure was significantly reduced by dealcoholized red wine at 7.4 mmHg and 5.4 mmHg by regular red wine. Resveratrol increases flow mediated vasodilation in a dose related manner, improves ED, prevents uncoupling of eNOS, increases adiponectin, lowers HS-CRP and blocks the effects of angiotensin II^[233-236]. The recommended dose is 250 mg/d of *trans* resveratrol^[234].

Lycopene

Lycopene is a fat-soluble phytonutrient in the carotenoid family. Dietary sources include tomatoes, guava, pink grapefruit, watermelon, apricots and papaya in high concentrations^[237-241]. Lycopene produces a significant reduction in BP, serum lipids and oxidative stress markers^[237-241]. Paran *et al*^[241] evaluated 30 subjects with Grade I hypertension, age 40-65, taking no antihypertensive or anti-lipid medications treated with a tomato lycopene extract (10 mg lycopene) for eight weeks. The SBP was reduced from 144 to 135 mmHg (9 mmHg reduction, $P < 0.01$) and DBP fell from 91 to 84 mmHg (7 mmHg reduction, $P < 0.01$). Another study of 35 subjects with Grade I hypertension showed similar results on SBP, but not DBP^[237]. Englehard gave a tomato extract to 31 hypertensive subjects over 12 wk demonstrating a significant BP reduction of 10/4 mmHg^[238]. Patients on various anti-hypertensive agents including ACEI, CCB and diuretics had a significant BP reduction of 5.4/3 mmHg over 6 wk when administered a standardized tomato extract^[239]. Other studies have not shown changes in BP with lycopene^[240]. Lycopene and tomato extract improve ED and reduce plasma total oxidative stress^[242]. The recommended daily intake of lycopene is 10-20 mg in food or supplement form.

Pycnogenol

Pycnogenol, a bark extract from the French maritime pine, at doses of 200 mg/d resulted in a significant reduction in SBP from 139.9 mmHg to 132.7 mmHg ($P < 0.05$) in eleven patients with mild hypertension over eight weeks in a double-blind randomized placebo crossover trial. Diastolic BP fell from 93.8 mmHg to 92.0 mmHg. Pycnogenol acts as a natural ACEI, protects cell membranes from oxidative stress, increases NO and improves endothelial function, reduces serum thromboxane concentrations, decreases myelo-peroxidase activity, improves renal cortical blood flow, reduces urinary albumin excretion and decreases HS-CRP^[243-247]. Other studies have shown reductions in BP and a decreased need for ACEI

and CCB, reductions in endothelin-1, HgA1C, fasting glucose, LDL-C and myeloperoxidase^[244,245,247].

Garlic

Clinical trials utilizing the correct dose, type of garlic and well absorbed long acting preparations have shown consistent reductions in BP in hypertensive patients with an average reduction in BP of 8.4/7.3 mmHg^[248,249]. Not all garlic preparations are processed similarly and are not comparable in antihypertensive potency^[1]. In addition, cultivated garlic (*allium sativum*), wild uncultivated garlic or bear garlic (*allium ursinum*) as well as the effects of aged, fresh and long acting garlic preparations differ^[1,70,71,248,249]. Garlic is also effective in reducing BP in patients with uncontrolled hypertension already on anti-hypertensive medication^[249,250]. A garlic homogenate-based supplement was administered to 34 prehypertensive and stage I hypertensive patients at 300 mg/d over 12 wk with a reduction in BP of 6.6-7.5/4.6-5.2 mmHg^[249]. Aged garlic at doses of 240 to 960 mg/d given to 79 hypertensive subjects over 12 wk significantly lowered SBP 11.8 ± 5.4 mmHg in the high dose garlic group^[249]. A time released garlic may reduce BP better than the shorter acting garlic^[249]. A Cochrane Database review indicated a net reduction in BP of 10-12/6-9 mmHg in all clinical trials with garlic^[249]. In a double-blind parallel randomized placebo-controlled trial of 50 patients, 900 mg of aged garlic extract with 2.4 mg of S-allylcysteine was administered daily for 12 wk and reduced SBP 10.2 mmHg ($P = 0.03$) more than the control group^[250].

Approximately 10000 mcg of allicin (one of the active ingredients in garlic) per day, the amount contained in four cloves of garlic (5 g) is required to achieve a significant BP lowering effect^[1,70,71,249,250]. Garlic has ACEI activity, calcium channel blocking activity, reduces catecholamine sensitivity, improves arterial compliance, increases bradykinin and nitric oxide and contains adenosine, magnesium, flavonoids, sulfur, allicin, phosphorous and ajoenes that reduce BP^[1,70,71].

Seaweed

Wakame seaweed (*Undaria pinnatifida*) is the most popular, edible seaweed in Japan^[251]. In humans, 3.3 g of dried Wakame for four wk significantly reduced both the SBP 14 ± 3 mmHg and the DBP 5 ± 2 mmHg ($P < 0.01$)^[252]. In a study of 62 middle-aged, male subjects with mild hypertension given a potassium-loaded, ion-exchanging, sodium-adsorbing, potassium-releasing seaweed preparation, significant BP reductions occurred at four weeks on 12 and 24 g/d of the seaweed preparation ($P < 0.01$)^[253]. The MAP fell 11.2 mmHg ($P < 0.001$) in the sodium-sensitive subjects and 5.7 mmHg ($P < 0.05$) in the sodium-insensitive subjects, which correlated with PRA.

Seaweed and sea vegetables contain most all of the seawater's 77I minerals and rare earth elements, fiber and alginate in a colloidal form^[251-253]. The primary effect of Wakame appears to be through its ACEI activity from at least four parent tetrapeptides and possibly their dipeptide

and tripeptide metabolites, especially those containing the amino acid sequence Val-Tyr, Ile-Tyr, Phe-Tyr and Ile-Try in some combination^[251,254,255]. Its long-term use in Japan has demonstrated its safety. Other varieties of seaweed may reduce BP by reducing intestinal sodium absorption and increasing intestinal potassium absorption^[253].

Sesame

Sesame has been shown to reduce BP in a several small randomized, placebo controlled human studies over 30-60 d^[256-264]. Sesame lowers BP alone^[257-261] or in combination with nifedipine^[256,260] diuretics and beta blockers^[257,261]. In a group of 13 mild hypertensive subjects, 60 mg of sesamin for 4 wk lowered SBP 3.5 mmHg ($P < 0.044$) and DBP 1.9 mmHg ($P < 0.045$)^[258]. Black sesame meal at 2.52 g/d over 4 wk in 15 subjects reduced SBP by 8.3 mmHg ($P < 0.05$) but there was a non-significant reduction in DBP of 4.2 mmHg^[259]. Sesame oil at 35 g/d significantly lowered central BP within 1 h and also maintained BP reduction chronically in 30 hypertensive subjects, reduced heart rate, reduced arterial stiffness, decreased augmentation index and pulse wave velocity, decreased HSCR, improved NO, decreased endothelin-I and improved antioxidant capacity^[264]. In addition sesame lowers serum glucose, HgbA1C and LDL-C, increases HDL, reduces oxidative stress markers and increases glutathione, SOD, GPx, CAT, vitamins C, E and A^[256,257,258-261]. The active ingredients are natural ACEI's such as sesamin, sesamol, sesaminol glucosides, furan-furan lignans which also suppressors of NF- κ B^[262,263]. All of these effects lower inflammation and oxidative stress, improve oxidative defense and reduce BP^[262,263].

Beverages: Tea, coffee, and cocoa

Green tea, black tea and extracts of active components in both have demonstrated reduction in BP in humans^[265-271]. In a double blind placebo controlled trial of 379 hypertensive subjects given green tea extract 370 mg/d for 3 mo, BP was reduced significantly at 4/4 mmHg with simultaneous decrease in HS CRP, TNF- α , glucose and insulin levels^[268].

Dark chocolate (100 g) and cocoa with a high content of polyphenols (30 mg or more) have been shown to significantly reduce BP in humans^[272-283]. A meta-analysis of 173 hypertensive subjects given cocoa for a mean duration of 2 wk had a significant reduction in BP 4.7/2.8 mmHg ($P = 0.002$ for SBP and 0.006 for DBP)^[276]. Fifteen subjects given 100 g of dark chocolate with 500 mg of poly-phenols for 15 d had a 6.4 mmHg reduction in SBP ($P < 0.05$) with a non significant change in DBP^[273]. Cocoa at 30 mg of poly-phenols reduced BP in pre-hypertensive and stage I hypertensive patients by 2.9/1.9 mmHg at 18 wk ($P < 0.001$)^[274]. Two more recent meta-analysis of 13 trials and 10 trials with 297 patients found a significant reduction in BP of 3.2/2.0 mmHg and 4.5/3.2 mmHg respectively^[276,279]. The BP reduction is the greatest in those with the highest baseline BP and those with at least 50%-70% cocoa at doses of 6 to 100 g/d^[280,282]. Cocoa may also improve insulin resistance and

endothelial function^[276,279,281].

Polyphenols, chlorogenic acids (CGAs), the ferulic acid metabolite of CGAs and di-hydro-caffeic acids decrease BP in a dose dependent manner, increase eNOS and improve endothelial function in humans^[284-286]. CGAs in green coffee bean extract at doses of 140 mg/d significantly reduced SBP and DBP in 28 subjects in a placebo-controlled randomized clinical trial. A study of 122 male subjects demonstrated a dose response in SBP and DBP with doses of CGA from 46 mg/d to 185 mg/d. The group that received the 185 mg dose had a significant reduction in BP of 5.6/3.9 mmHg ($P < 0.01$) over 28 d. Hydroxyhydroquinone is another component of coffee beans which reduces the efficacy of CGAs in a dose-dependent manner which partially explains the conflicting results of coffee ingestion on BP^[284,286]. Furthermore, there is genetic variation in the enzyme responsible for the metabolism of caffeine modifies the association between coffee intake, amount of coffee ingested and the risk of hypertension, heart rate, MI, arterial stiffness, arterial wave reflections and urinary catecholamine levels^[287]. Fifty-nine percent of the population has the I F/ I A allele of the CYP1A2 genotype which confers slow metabolism of caffeine. Heavy coffee drinkers who are slow metabolizers had a 3.00 HR for developing hypertension. In contrast, fast metabolizers with the I A/ I A allele have a 0.36 HR for incident hypertension^[288].

Additional compounds

Melatonin demonstrates significant anti-hypertensive effects in humans in a numerous double-blind randomized placebo controlled clinical trials at 3-5 mg/d^[289-299]. The average reduction in BP is 6/3 mmHg. Melatonin stimulates GABA receptors in the CNS and vascular melatonin receptors, inhibits plasma A II levels, improves endothelial function, increases NO, vasodilates, improves nocturnal dipping, lowers cortisol and is additive with ARBs. Beta blockers reduce melatonin secretion^[300].

Hesperidin significantly lowered DBP 3-4 mmHg ($P < 0.02$) and improved microvascular endothelial reactivity in 24 obese hypertensive male subjects in a randomized, controlled crossover study over 4 wk for each of three treatment groups consuming 500 mL of orange juice, hesperidin or placebo^[301].

Pomegranate juice is rich in tannins and has numerous other properties that improve vascular health and reduces the SBP by 5%-12%^[302,303]. A study of 51 healthy subjects given 330 mg/d of pomegranate juice had reduction in BP of 3.14/2.33 mmHg ($P < 0.001$)^[303]. Pomegranate juice also suppresses the postprandial increase in SBP following a high-fat meal^[303]. Pomegranate juice reduces serum ACE activity by 36%, and has anti-atherogenic, antioxidant and anti-inflammatory effects^[302,303]. Pomegranate juice at 50 mL/d reduced carotid IMT by 30% over one year, increased PON 83%, decreased oxLDL by 59%-90%, decreased antibodies to oxLDL by 19%, increased total antioxidant status by 130 %, reduced TGF- β , increased catalase, SOD and GPx, increased eNOS and NO and improved endothelial function^[304,305].

Pomegranate juice works like an ACEI.

Grape seed extract (GSE) was administered to subjects in nine randomized trials, meta-analysis of 390 subjects and demonstrated a significant reduction in SBP of 1.54 mmHg ($P < 0.02$)^[304,305]. Significant reduction in BP of 11/8 mmHg ($P < 0.05$) were seen in another dose response study with 150 to 300 mg/d of GSE over 4 wk^[306]. GSE has high phenolic content which activates the PI3K/Akt signaling pathway that phosphorylates eNOS and increases NO^[306,307].

Coenzyme Q10 (Ubiquinone)

Coenzyme Q10 has consistent and significant antihypertensive effects in patients with essential hypertension^[1,308-317]. The literature is summarized below: (1) Compared to normotensive patients, essential hypertensive patients have a higher incidence (6 fold) of coenzyme Q10 deficiency documented by serum levels^[1]; (2) Doses of 120 to 225 mg/d of coenzyme Q10, depending on the delivery method or the concomitant ingestion with a fatty meal, are necessary to achieve a therapeutic level of 3 ug/mL^[1,313,314]. This dose is usually 3-5 mg/kg per day of coenzyme Q10. Oral dosing levels may become lower with nanoparticle and emulsion delivery systems intended to facilitate absorption^[315]. Adverse effects have not been characterized in the literature; (3) Patients with the lowest coenzyme Q10 serum levels may have the best antihypertensive response to supplementation; (4) The average reduction in BP is about 15/10 mmHg and heart rate falls 5 beats/min based on reported studies and meta-analysis; (5) The antihypertensive effect takes time to reach its peak level at 4 wk. Then the BP remains stable during long term treatment. The antihypertensive effect is gone within two weeks after discontinuation of coenzyme Q10. The reduction in BP and SVR are correlated with the pretreatment and post treatment serum levels of coenzyme Q10. About 50% of patients respond to oral coenzyme Q10 supplementation for BP^[309]; (6) Approximately 50% of patients on antihypertensive drugs may be able to stop between one and three agents. Both total dose and frequency of administration may be reduced. (7) Patients administered coenzyme Q10 with enalapril improved the 24 h ABM better than with enalapril monotherapy and also normalized endothelial function^[310]; and (8) Coenzyme Q10 is a lipid phase antioxidant and free radical scavenger, increases eNOS and NO, reduces inflammation and NF- κ B and improves endothelial function and vascular elasticity^[1,311,312].

Other favorable effects on cardiovascular risk factors include improvement in the serum lipid profile and carbohydrate metabolism with reduced glucose and improved insulin sensitivity, reduced oxidative stress, reduced heart rate, improved myocardial LV function and oxygen delivery and decreased catecholamine levels^[1,311,312].

Alpha lipoic acid

Alpha lipoic acid (ALA) is known as thioctic acid in Europe where it is a prescription medication. It is a sulfur-containing compound with antioxidant activity both in

water and lipid phases^[1,70,71]. Its use is well-established in the treatment of certain forms of liver disease and in the delay of onset of peripheral neuropathy in patients with diabetes. Recent research has evaluated its potential role in the treatment of hypertension, especially as part of the MS^[318-321]. In a double-blind cross over study of 36 patients over 8 wk with CHD and hypertension, 200 mg of lipoic acid with 500 mg of acetyl-L-carnitine significantly reduced BP 7/3 mmHg and increased brachial artery diameter^[320]. The QUALITY study of 40 patients with DM and stage I hypertension showed significant improvements in BP, urinary albumin excretion, FMD and insulin sensitivity over 8 wk with a combination of Quinapril (40 mg/d) and lipoic acid (600 mg/d) that was greater than either alone^[320]. Lipoic acid increases levels of glutathione, cysteine, vitamin C and vitamin E, inhibits NF- κ B, reduces endothelin-1, tissue factor and VCAM-1, increases cAMP, downregulates CD4 immune expression on mononuclear cells, reduces oxidative stress, inflammation, reduces atherosclerosis in animal models, decreases serum aldehydes and closes calcium channels which improves vasodilation, increases NO and nitrosothiols, improves endothelial function and lowers BP^[1,318-321]. Lipoic acid normalizes membrane calcium channels by providing sulfhydryl groups, decreasing cytosolic free calcium and lowers SVR. In addition, lipoic acid improves insulin sensitivity which lowers glucose and advanced glycosylation end products which improves BP control and lowers serum triglycerides. Morcos *et al*^[321], showed stabilization of urinary albumin excretion in DM subjects given 600 mg of ALA compared to placebo for 18 mo ($P < 0.05$).

The recommended dose is 100 to 200 mg/d of R-lipoic acid with biotin 2-4 mg/d to prevent biotin depletion with long term use of lipoic acid. R-lipoic acid is preferred to the L isomer because of its preferred use by the mitochondria^[1,32,71].

NAC: NAC and L arginine (ARG) in combination reduce endothelial activation and BP in hypertensive patients with type 2 DM^[141]. Over 6 mo 24 subjects given placebo or NAC with ARG, significantly reduced both systolic and diastolic BP ($P = 0.05$)^[141]. In addition, ox LDL, HSCRP, ICAM, VCAM, fibrinogen and PAI-1 were decreased while HDL, NO and endothelial postischemic vasodilation increased^[141]. NAC increases NO *via* IL-1b and increases iNOS mRNA, increases glutathione by increasing cysteine levels, reduces the affinity for the AT1 receptor by disrupting disulfide groups, blocks the L type calcium channel, lowers homocysteine, and improves IMT^[141,322-324]. The recommended dose is 500 to 1000 mg bid.

Hawthorne extract has been used for centuries for the treatment of hypertension, CHF and other cardiovascular diseases^[325-329]. A recent four-period crossover design, dose response study in 21 subjects with prehypertension or mild hypertension over 3^{1/2} d, did not show changes in FMD or BP on standardized extract with 50 mg of oligomeric procyanidin per 250 mg extract with 1000 mg, 1500 or 2500 mg of the extract^[325]. Hawthorne showed non inferiority of ACEI and diuretics in the treatment of

Table 4 Integrative approach to the treatment of hypertension

Intervention category	Therapeutic intervention	Daily intake
Diet characteristics	DASH I , DASH II-Na ⁺ or premier diet	Diet type
	Sodium restriction	1500 mg
	Potassium	5000 mg
	Potassium/sodium ratio	> 3:1
	Magnesium	1000 mg
	Zinc	50 mg
Macronutrients	Protein total intake from non-animal sources, organic lean or wild animal protein, or coldwater fish	30% of total calories, which 1.5-1.8 g/kg body weight
	Whey protein	30 g
	Soy protein (fermented sources are preferred)	30 g
	Sardine muscle concentrate extract	3 g
	Milk peptides	30-60 mg
	Fat	30% of total calories
	Omega-3 fatty acids	2-3 g
	Omega-6 fatty acids	1 g
	Omega-9 fatty acids	2-4 tablespoons of olive or nut oil or 10-20 olives
	Saturated fatty acids from wild game, bison, or other lean meat	< 10% total calories
	Polyunsaturated to saturated fat ratio	< 2.0
	Omega 3 to omega 6 ratio	1.1-1.2
	Synthetic trans fatty acids	None (completely remove from diet)
	Nuts in variety	Ad libitum
	Carbohydrates as primarily complex carbohydrates and fiber	40% of total calories
	Oatmeal or	60 g
	Oatbran or	40 g
	Beta-glucan or	3 g
	Psyllium	7 g
Specific foods	Garlic as fresh cloves or aged kyolic garlic	4 fresh cloves (4 g) or 600 mg aged garlic taken twice daily
	Sea vegetables, specifically dried wakame	3.0-3.5 g
	Lycopene as tomato products, guava, watermelon, apricots, pink grapefruit, papaya or supplements	10-20 mg
	Dark chocolate	100 g
	Pomegranate juice or seeds	8 ounces or one cup
	Sesame	60 mg sesamin or 2.5 g sesame meal
Exercise	Aerobic	20 min daily at 4200 kJ/wk
	Resistance	40 min/d
Weight reduction	Body mass index < 25	Lose 1-2 pounds per week and increasing the proportion of lean muscle
	Waist circumference:	
	< 35 inches for women	
	< 40 inches for men	
	Total body fat:	
Other lifestyle recommendations	< 22% for women	
	< 16% for men	
	Alcohol restriction:	< 20 g/d
	Among the choice of alcohol red wine is preferred due to its vasoactive phytonutrients	Wine < 10 ounces
		Beer < 24 ounces
		Liquor < 2 ounces
Medical considerations	Caffeine restriction or elimination depending on CYP 450 type	< 100 mg/d
	Tobacco and smoking	Stop
	Medications which may increase blood pressure.	Minimize use when possible, such as by using disease-specific nutritional interventions
		100-200 mg twice daily
Supplemental foods and nutrients	Alpha lipoic acid with biotin	
	Amino acids:	
	Arginine	5 g twice daily
	Carnitine	1 to 2 g twice daily
	Taurine	1 to 3 g twice daily
	Chlorogenic acids	150-200 mg
	Coenzyme Q10	100mg once to twice daily
	Grape seed extract	300 mg
	Hawthorne extract	500 mg twice a day
	Melatonin	2.5 mg
	N-acetyl cysteine	500 mg twice a day
	Olive leaf extract (oleuropein)	500 mg twice a day
	Pycnogenol	200 mg
	Quercetin	500 mg twice a day

Resveratrol (<i>trans</i>)	250 mg
Vitamin B6	100 mg once to twice daily
Vitamin C	250-500 mg twice daily
Vitamin D3	Dose to raise 25-hydroxyvitamin D serum level to 60 ng/mL
Vitamin E as mixed tocopherols	400 IU

102 patients with NYHC II CHF over 8 wk^[327]. Patients with hypertension and type 2 DM on medications for BP and DM were randomized to 1200 mg of hawthorne extract for 16 wk showed significant reductions in DBP of 2.6 mmHg ($P = 0.035$)^[328]. Thirty six mildly hypertensive patients were administered 500 mg of hawthorne extract for 10 wk and showed a non significant trend in DBP reduction ($P = 0.081$) compared to placebo^[329]. Hawthorne acts like an ACEI, BB, CCB and diuretic. More studies are needed to determine the efficacy, long term effects and dose of hawthorne for the treatment of hypertension.

Quercetin is an antioxidant flavonol found in apples, berries and onions that reduces BP in hypertensive individuals^[330,331] but the hypotensive effects do not appear to be mediated by changes in HSCR, TNF- α , ACE activity, ET-1, NO, vascular reactivity or FMD^[330]. Quercetin is metabolized by CYP 3A4. Quercetin was administered to 12 hypertensive men at an oral dose of 1095 mg with reduction in mean BP by 5 mmHg, SBP by 7 mmHg and DBP by 3 mmHg^[330]. The maximal plasma level at 10 h was $2.3 \pm 1.8 \mu\text{mol/L}$, with return to baseline levels at 17 h. Forty one pre-hypertensive and stage I hypertensive subjects were enrolled in a randomized, double-blind, placebo-controlled, crossover study with 730 mg of quercetin per day *vs* placebo^[331]. In the stage I hypertensive patients, the BP was reduced by 7/5 mmHg ($P < 0.05$) but there were no changes in oxidative stress markers^[331]. Quercetin administered to 93 overweight or obese subjects at 150 mg/d (plasma levels of 269 nmol/L) over 6 wk lowered SBP 2.9 mmHg in the hypertensive group and up to 3.7 mmHg in SBP in the patients 25-50 years of age^[332]. The recommended dose of quercetin is 500 mg bid.

CLINICAL CONSIDERATIONS

Combining food and nutrients with medications

Several of the strategic combinations of nutraceutical supplements together or with anti-hypertensive drugs, have been shown to lower BP more than the medication alone: (1) Sesame with beta blockers, diuretics and nifedipine; (2) Pycnogenol with ACEI and CCB; (3) Lycopene with ACEI, CCB and diuretics; (4) ALA with ACEI or acetyl-L Carnitine; (5) Vitamin C with CCB's; (6) NAC with arginine; (7) Garlic with ACEI, diuretics and beta blockers; (8) Coenzyme Q10 with ACEI and CCB; (9) Taurine with magnesium; (10) Potassium with all anti-hypertensive agents; and (11) Magnesium with all anti-hypertensive agents.

Many anti-hypertensive drugs may cause nutrient depletions that can actually interfere with their anti-hypertensive action or cause other metabolic adverse effects

manifest through the lab or with clinical symptoms^[333]. Diuretics decrease potassium, magnesium, phosphorous, sodium, chloride, folate, vitamin B6, zinc, iodine and coenzyme Q10; increase homocysteine, calcium and creatinine; and elevate serum glucose by inducing insulin resistance. Beta blockers reduce coenzyme Q10. ACEI and ARB's reduce zinc.

Vascular biology such as endothelial and VSMD plays a primary role in the initiation and perpetuation of hypertension, CVD and TOD. Nutrient-gene interactions and epigenetics are a predominant factor in promoting beneficial or detrimental effects in cardiovascular health and hypertension. Food and nutrients can prevent, control and treat hypertension through numerous vascular biology mechanisms. Oxidative stress, inflammation and autoimmune dysfunction initiate and propagate hypertension and cardiovascular disease. There is a role for the selected use of single and component nutraceutical supplements vitamins, antioxidants and minerals in the treatment of hypertension based on scientifically controlled studies as a complement to optimal nutritional, dietary intake from food and other lifestyle modifications^[333]. A clinical approach which incorporates diet, foods, nutrients, exercise, weight reduction, smoking cessation, alcohol and caffeine restriction, and other lifestyle strategies can be systematically and successfully incorporated into clinical practice (Table 4).

CONCLUSION

Vascular biology, endothelial and vascular smooth muscle and cardiac dysfunction play a primary role in the initiation and perpetuation of hypertension, cardiovascular disease and TOD. Nutrient-gene interactions and epigenetics are predominant factors in promoting beneficial or detrimental effects in cardiovascular health and hypertension. Macronutrients and micronutrients can prevent, control and treat hypertension through numerous mechanisms related to vascular biology.

Oxidative stress, inflammation and autoimmune dysfunction initiate and propagate hypertension and cardiovascular disease. There is a role for the selected use of single and component nutraceutical supplements, vitamins, antioxidants and minerals in the treatment of hypertension based on scientifically controlled studies which complement optimal nutrition, coupled with other lifestyle modifications.

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Therapeutic interventions for heart failure with preserved ejection fraction: A summary of current evidence

Muhammad Asrar ul Haq, Chiew Wong, Vivek Mutha, Nagesh Anavekar, Kwang Lim, Peter Barlis, David L Hare

Muhammad Asrar ul Haq, Chiew Wong, Vivek Mutha, Nagesh Anavekar, Peter Barlis, Department of Cardiology, The Northern Hospital, Epping, VIC 3076, Australia
Muhammad Asrar ul Haq, Chiew Wong, Vivek Mutha, Nagesh Anavekar, Kwang Lim, Peter Barlis, David L Hare, Department of Medicine, University of Melbourne, Melbourne 3010, Australia

Author contributions: All authors contributed to this paper equally.

Correspondence to: Dr. Muhammad Asrar ul Haq, MBBS, Department of Cardiology, The Northern Hospital, 185 Cooper Street, Epping, VIC 3076, Australia. asrar.ulhaq@me.com
Telephone: +61-3-80458000 Fax: +61-3-80458045

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(HFPEF) is common and represents a major challenge in cardiovascular medicine. Various pharmacological interventions available for heart failure with reduced ejection fraction have not been supported by clinical studies for HFPEF. This article presents a brief overview of the currently recommended therapeutic strategies for HFPEF.

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Abstract

Heart failure with preserved ejection fraction (HFPEF) is common and represents a major challenge in cardiovascular medicine. Most of the current treatment of HFPEF is based on morbidity benefits and symptom reduction. Various pharmacological interventions available for heart failure with reduced ejection fraction have not been supported by clinical studies for HFPEF. Addressing the specific aetiology and aggressive risk factor modification remain the mainstay in the treatment of HFPEF. We present a brief overview of the currently recommended therapeutic options with available evidence.

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Key words: Heart failure; Diastolic dysfunction; Heart failure with preserved ejection fraction; Heart failure with normal ejection fraction

Core tip: Heart failure with preserved ejection fraction

INTRODUCTION

Prevalence of diastolic heart failure (HF) has been rising steadily in the recent past. It is now well established that at least half of patients presenting with symptoms and signs of HF have preserved left ventricular (LV) ejection fraction, *i.e.*, heart failure with preserved ejection fraction (HFPEF), and that this portion of the HF population consists predominantly of women, older age group, and people with hypertension and other cardiovascular risk factors^[1-3]. The prevalence of HFPEF varies from 1.1%-5.5%, depending on the age and other variables, *e.g.*, diagnostic criteria and methods, and rises to 3.1%-5.5% when studies are confined to a older population aged 65 years or above^[6-9]. Chronic hypertension is the most common cause in addition to age, with suggestion of up to 60% of patients with HFPEF being hypertensive^[10,11]. Obesity and Diabetes also contribute independently to the development of diastolic dysfunction^[12-15]. Other conditions associated with diastolic dysfunction are Coronary artery disease and hypertrophic or restrictive cardiomyopathies.

It is observed that the morbidity and mortality associated with HFPEF is much higher than the normal population^[16]. Several studies have reported an annual mortality rate ranging from 5% to 8% in this population^[17-19], much higher than the age-matched controls^[20-22]. Given the accumulated data of various studies, it appears that all-cause mortality of HF patients in the community is similar whether their contractility is preserved or not.

Most of the current treatment of HFPEF is based on morbidity benefits and symptom reduction. Various pharmacological interventions available for heart failure with reduced ejection fraction (HFREF) have not been supported by clinical studies for HFPEF. Addressing the specific aetiology and aggressive risk factor modification remain the mainstay in the treatment of HFPEF. Current guidelines recommend the management should involve treatment of hypertension, control of heart rate, venous pressure reduction, and prevention of myocardial ischemia^[23-25]. Here we present a brief overview of the currently recommended therapeutic options with available evidence.

TREATING THE HYPERTENSION

Treatment of hypertension remains one of the most important factors in the management of diastolic dysfunction^[23,25]. Effective management of increased blood pressure can reduce left atrial and LV end diastolic pressures, and enhance the LV filling by improving relaxation. It can further benefit by reduction of LV hypertrophy (LVH) and hence reducing the risk of development or progression of HF. Studies of hypertensive subjects indicate that diastolic dysfunction improves with LVH regression^[26]. Angiotensin converting enzyme inhibitors (ACEi) inhibitors or aldosterone antagonists such as spironolactone can have protective effect against the exaggerated fibrous tissue response^[27,28]. Thus theoretically, there may be benefits to inhibit renin-angiotensin-aldosterone system (RAAS) beyond blood pressure reduction.

In the Systolic Hypertension in the Elderly Program study^[29], a good control of isolated systolic hypertension with chlorthalidone and atenolol in a population of 4736 patients aged 60 years and older during an average of 4.5 years of follow-up led to significant reduction in the risk of HF {55 *vs* 105 in placebo group; RR = 0.51; 95%CI: 0.37-0.71, *P* < 0.001; number needed to treat to prevent 1 event [number needed to treat (NNT)], 48} and LV mass index, by 13%. In particular, among patients with prior MI, an 80% risk reduction was observed.

The Valsartan In Diastolic Dysfunction^[30] studied the effects of blood pressure reduction on the myocardial relaxation on Doppler tissue imaging after a 38 wk of exposure to different anti hypertensive agents, including renin-angiotensin system inhibitor Valsartan in one group matched with placebo in the other. The difference in blood pressure reduction between the two groups was not significant ($12.8 \pm 17.2/7.1 \pm 9.9$ mmHg reduction in the valsartan group *vs* $9.7 \pm 17.0/5.5 \pm 10.2$ mmHg in the placebo group). Diastolic relaxation velocity was in-

creased by 0.60 ± 1.4 cm/s from baseline in the valsartan group (*P* < 0.0001) and 0.44 ± 1.4 cm/s from baseline in the placebo group (*P* < 0.0001) by week 38. However, there was no significant difference in the change in diastolic relaxation velocity between the two groups (*P* = 0.29). This suggested that lowering blood pressure improves diastolic function irrespective of the type of antihypertensive agent used.

Effects of blood pressure reduction on LVH have also been studied. Beta-blockers and diuretics are well established interventions for prevention of cardiovascular morbidity and death in patients with hypertension. In the Losartan Intervention For Endpoint reduction in hypertension study^[31], regression of LVH after a year of anti-hypertensive therapy was associated with improvement of various LV diastolic filling parameters on echocardiography. In this trial, Dahlöf *et al*^[31] demonstrated superiority of an angiotensin receptor blocker (ARB), losartan, to β -blockade in reducing the composite primary endpoint (cardiovascular death, myocardial infarction or stroke; *P* = 0.021) and in regression of LVH (*P* < 0.0001), suggesting that besides blood pressure reduction, blockade of the AT1 receptor by losartan offers additional benefits for cardiovascular morbidity and mortality as compared to β -blockade, for a similar reduction in blood pressure, and was better tolerated.

The Candesartan in Heart failure: Assessment of Reduction in Mortality and morbidity-Preserved (CHARM-Preserved) trial^[32] comparing the effects of candesartan *vs* placebo in HFPEF (EF > 40%) in 3023 patients (1514 in candesartan and 1509 in placebo group) reported a moderate impact of candesartan in preventing admissions for HF (230 *vs* 279, *P* = 0.017) over a period of 36.6 mo. There was however no difference in mortality between the two groups (170 *vs* 170 cardiovascular deaths). Similar results were observed in Perindopril In Elderly People With Chronic Heart Failure (PEP-CHF) trial^[33] in which a total of 850 patients aged ≥ 70 with HFPEF were randomized to perindopril 4 mg or placebo. The mean follow up period was 26.2 mo. In the first year of treatment, the hospitalizations for HF were less frequent in the perindopril group (*P* = 0.033), and significant improvement in the New York Heart Association (NYHA) class and functional capacity on 6-min walk test was observed in patients receiving perindopril (*P* < 0.030), however the mortality rate in both groups was similar. This study had insufficient power for its primary endpoint, which may be attributable to the non significant results of perindopril effects on long-term (> 1 year) morbidity and mortality of these patients. Differential Effects of Antihypertensive Treatment on LV Diastolic Function^[34] suggested that patients receiving treatment with an amlodipine-based regimen had better diastolic function than patients treated with the atenolol-based regimen, independent of blood pressure reduction and other factors that are known to affect diastolic function.

It has been suggested that aggressive blood pressure lowering with a combination of an ARB, valsartan; a calcium channel blocker (CCB), amlodipine; and potential

additional therapy with diuretics or β -blockers was associated with improved annular relaxation velocity (e') on tissue doppler imaging, a measure of diastolic function, in patients with hypertension and diastolic dysfunction^[35]. In this study, the patients who achieved the greatest blood pressure reduction had the best improvement in diastolic function, which supports that lower blood pressure targets may be an effective means to improve this measure of myocardial target-organ damage in hypertension.

CONTROLLING THE HEART RATE

Tachycardia is poorly tolerated in the presence of diastolic dysfunction and the guidelines recommend beta-blockers or CCB for decreasing heart rate^[23]. These drugs may also be helpful in stabilising rhythm and preventing atrial arrhythmias [*e.g.*, atrial fibrillation (AF)], which can cause substantial increase in diastolic and atrial filling pressures, leading to abrupt hemodynamic deterioration due to loss of the atrial contribution to diastolic filling. AF is common in HFPEF patients with a prevalence of up to 41%^[36] and a recent meta-analysis^[37] of 16 studies for the prognostic significance of AF in HF involving 53969 patients suggested that the presence of AF is associated with an adverse prognosis in HF irrespective of LV systolic function.

The diastole accounts for nearly 70% of the cardiac cycle at a heart rate of 60 bpm, slightly over 50% at 120 bpm, and only 40% at 180 bpm. The LV filling time is therefore considerably shortened with increased heart rate because the relaxation between beats is incomplete. In addition, in people with HFPEF tachycardia results in delayed relaxation and increased diastolic pressure. Things get further complicated during exercise. In patients with HFPEF, the heart is unable to take advantage of the Frank-Sterling mechanism during exercise. A stiff ventricle, despite elevated filling pressure, does not increase in volume. As a consequence filling pressure increases but cardiac output does not.

Therefore, decreasing heart rate would result in reduced pressure in the early period of the diastole by improving relaxation. Similarly increasing the ventricular filling time would improve cardiac output, and reduce symptoms during exercise.

The Study of Effects of Nebivolol Intervention on Outcomes and Rehospitalisation in Seniors With Heart Failure (SENIORS) demonstrated that nebivolol reduces the composite risk of all-cause mortality and cardiovascular hospital admission in elderly patients with chronic HF and, importantly, that ejection fraction does not influence the clinical effects of nebivolol^[38]. This trial randomized 112 patients in 29 European centres, of whom 104 were evaluable for the study; 43 with EF \leq 35% and 61 with an EF $>$ 35%. LV end-systolic volume (ESV), EF, mitral valve E/A ratio, and E-wave deceleration time were assessed at baseline and after 12 mo. In the group with EF \leq 35%, nebivolol reduced ESV and improved EF; no changes were observed in the E/A ratio or E-wave deceleration time. In EF $>$ 35% group, no significant changes

in either systolic or diastolic parameters were observed. This absence of detectable changes with standard echocardiography in patients with predominant diastolic HF questions the mechanism of benefit on morbidity/mortality in this population. In the separate analysis of patients with an EF cut off greater than 40%, there was no noted statistical interaction, suggesting that nebivolol was of comparable benefit in reduced EF and preserved EF patients.

VENOUS PRESSURE REDUCTION

Diuretics remain the mainstay of symptomatic treatment for venous congestion similar to the management of systolic dysfunction. However, in patients with HFPEF optimising the volume status may be complicated by a narrow therapeutic margin given that in this group of patients the pressure/volume curve differs from the physiological curve and even a small decrease in filling pressure can result in a marked reduction of LV diastolic volume, which may lead to a significant reduction of cardiac output, risk of hypotension and renal impairment^[39,40]. The doses of diuretics in this group of patients are therefore much lower than those in patients with systolic dysfunction. Diuretics do not directly affect the myocardium, while nitrates improve the ability of the left ventricle to increase its volume by releasing nitric oxide (NO).

Spironolactone combines diuretic action with beneficial effects on the structure of the left ventricle. The results of Treatment of Preserved Cardiac Function Heart failure with an Aldosterone Antagonist^[41] was however a negative study, failing to show benefit for the clinical composite primary end point despite significantly fewer heart-failure hospitalisations, a part of the primary end point, over the average follow-up of 3.3 years. Aldosterone Receptor Blockade in Diastolic Heart Failure study^[42] suggested that long-term aldosterone receptor blockade with spironolactone improved diastolic function but did not affect clinical symptoms or exercise capacity. Therefore, further investigation into the clinical significance of these echocardiographic findings will be required in larger studies.

PREVENTION OF MYOCARDIAL ISCHEMIA

Myocardial ischemia is one of the most important mechanisms underlying HFPEF. Improved myocardial oxygen balance leads to better LV relaxation, reduced LVEDP, reduced risk of cardiac arrhythmias and stabilises the heart rate. It is therefore vital to use drugs that reduce oxygen consumption by the myocardium (beta-blockers, CCB, nitrates) and revascularization to improve oxygen supply to the myocardium. Flash pulmonary oedema frequently reoccurs in association with marked systolic hypertension, even after coronary revascularisation, suggesting that control of hypertension is important and that coronary revascularisation may not be adequate to

prevent reoccurrence of flash pulmonary oedema^[43].

SPECIFIC THERAPEUTIC AGENTS

Given the limited evidence regarding directed therapy for HFPEF, treatment of factors known to exacerbate diastolic dysfunction plays a vital role. All patients with diastolic dysfunction should get adequately treated for associated conditions, *i.e.*, diabetes, obesity, primary myocardial disease, or pericardial disease in addition to above mentioned hypertension, myocardial ischemia.

ACE inhibitors

The theoretical benefits of ACE inhibitors specifically in HFPEF rest on the basis that angiotensin II contributes to LV myocardial hypertrophy and fibrosis, impairs LV relaxation, and increases the stiffness of the left ventricle^[44]. All of these factors, potentially improved by ACE inhibitors, will therefore improve diastolic function. Clinical studies evaluating ACE inhibitors in HFPEF have shown contradicting results. Secondary endpoints of reduced hospitalisation and improved exercise tolerance has been suggested by few^[33,45] while other studies demonstrated no benefit except in patients with previous myocardial infarction^[46].

A small study assessed the effect of enalapril on 21 elderly patients with HFPEF (LVEF > 50%) and history of myocardial infarction^[45]. These patients had received furosemide for 2 wk or greater before the initiation of the study, and were on a constant dose of furosemide, were randomized to receive enalapril, titrated up to 20 mg daily as tolerated, and followed for 3 mo. There was a significant difference from baseline to study termination in the study outcomes in the treatment group: NYHA class improved from 3 to 2.4 ($P = 0.005$) and exercise time with the modified Bruce protocol increased from 224 to 270 s, versus no significant difference in the placebo group. Another small prospective study in France enrolled 358 subjects who were admitted for a first episode of HF but had ejection fractions $\geq 50\%$. Patients were separated into 2 groups based on whether or not they were prescribed an ACEi at discharge; lisinopril (32.3%), ramipril (25.6%), perindopril (23.8%), or enalapril (5.5%). The authors attempted to adjust for selection bias by developing a propensity score and comparing matched controls. Patients who had been prescribed ACEIs had a 10% reduction in 5-year mortality (NNT = 10). The largest and most well-designed study (PEP-CHF)^[33] demonstrated no significant difference in the primary endpoint but showed significant reduction in the secondary endpoint of hospitalization for HF.

ARB

Although well understood association of RAAS with many of the underlying processes behind HFPEF, various studies involving ARB use in HFPEF did not demonstrate significant benefits, except CHARM-Preserved, which relates reduced hospitalization with candesartan^[32].

Irbesartan in Patients with Heart Failure and Preserved Ejection Fraction trial compared irbesartan with placebo I 4128 patients with HFPEF^[47]. It did not improve the outcomes of patients.

β -blockers

The mechanism behind β -blockers' potential in improving diastolic function in patients with HFPEF is believed to be associated with the drugs' negative chronotropic and inotropic properties in stabilising the heart rate and helping the ventricle to relax^[44]. SENIORS study was the largest trial evaluating the effect of nebivolol on the composite of all-cause mortality or hospitalization for a cardiovascular cause^[48], which reported that nebivolol, a beta-blocker with vasodilation properties, is an effective and well-tolerated treatment for HF in the elderly. The subgroup with ejection fraction > 35% was analysed in a pre-specified analysis. The interaction test showed that ejection fraction did not modify the effect of nebivolol in terms of the primary outcome (all-cause mortality or cardiovascular hospitalization) (HR = 0.86; 95%CI: 0.74-0.99, $P = 0.039$ in the main analysis), implying that the effect of nebivolol is similar in patients with HF and an ejection fraction $\leq 35\%$ and > 35%. However, when the ejection fraction threshold of 40% was used instead of 35% (which was not a pre-specified subgroup), there was no significant difference between those treated with nebivolol and those given placebo (HR = 0.83; 95%CI: 0.62-1.11, $P = 0.203$).

Another study, conducted in elderly patients (mean age 81 years), enrolled patients 62 years and older with NYHA class II or III HF, prior Q-wave MI, and EF $\geq 40\%$ who had also been on ACE inhibitors and diuretics for 2 mo^[49]. This trial analysed the effect of propranolol on all-cause mortality and the composite of all-cause mortality and nonfatal MI after a follow-up period of 32 mo. All patients were on ACE inhibitors and diuretics during the study and digoxin was administered only in cases of atrial fibrillation. There was a significant difference between the groups in all-cause mortality (56% *vs* 76%; $P = 0.007$) and all-cause mortality plus nonfatal myocardial infarction (59% *vs* 82%; $P = 0.002$) favouring the patients treated with propranolol compared with those patients who were only on conventional therapy and no propranolol. The reduction in total mortality began 1 year after treatment initiation and the beneficial effects lasted until the end of the study. However, the percentage of deaths due to cardiac causes in each group did not differ significantly. Overall, studies that assessed the role of β -blockers in HFPEF have all found β -blockers to positively impact study outcomes (mortality in post myocardial infarction patients specifically and morbidity in others).

Digoxin

The use of digoxin has beneficial effect on hospitalization in HFPEF^[50]. This effect however has not been shown in HFPEF^[51]. Furthermore, digoxin has not shown any

impact on mortality in either HFREF or HFPEF.

OVERALL EFFECTS OF COMBINED PHARMACOTHERAPY ON EXERCISE TOLERANCE, CARDIAC FUNCTION, AND MORTALITY IN HFPEF

A recent meta-analysis was sought to determine whether pharmacologic interventions changed exercise capacity, diastolic function, and mortality in HFPEF^[52]. Data from 53878 patients enrolled in 30 published reports were collated including 18 randomized controlled trials ($n = 11253$) and 12 observational studies ($n = 42625$). A combined pharmacotherapy for HFPEF demonstrated a quantifiable improvement in exercise tolerance but failed to show a mortality benefit.

ROLE OF EXERCISE TRAINING

Exercise training is now widely used as an adjunct therapy for the stable HF patient. It is recommended by the American College of Cardiology and the American Heart Association at a Class 1 level^[24]. Many physical activity benefits for HF patients have been documented, such as improvements in physical capacity (an increase of 10%-30% of the maximum physical capacity)^[53,54], improvements in quality of life^[55], endothelial dysfunction^[56], circulating catecholamine levels^[57], morbidity and hospital admissions^[58]. However most of the studies have focused on patients with HFREF. Since the patients with HFPEF also experience exercise intolerance, dyspnoea, early fatigue, and similar mortality risk and re-hospitalization rates, a case can be made for exercise to be part of the management of people with HFPEF. In a recent study, 3 years of exercise-based lifestyle intervention was not effective in reducing progression of subclinical diastolic dysfunction in patients with type 2 diabetes mellitus^[59]. Other studies have suggested an improvement in exercise tolerance, quality of life and depression scale with low-to-moderate intensity exercise^[60-62].

Effects of exercise training on LV diastolic function in patients with systolic dysfunction have included a significant reduction in LV diastolic wall stress at low work rates resulting in a 30% increase in peak oxygen consumption after 2 mo^[63]. Belardinelli *et al.*^[64] evaluated patients with dilated cardiomyopathy and a Doppler mitral inflow profile suggestive of concomitant abnormal diastolic LV function. Only people with delayed relaxation improved their functional capacity after training. In these patients, the diastolic filling pattern normalised after training. Those with a restrictive filling pattern, however, were found to have a worse prognosis and did not improve functional capacity or diastolic filling pattern after training.

The standard recommendations for exercise training in general include aerobic activity performed at least 30 min, 5 or more days/week. Exercise intensity in HF train-

ing has varied between studies, and some study protocols have used interval or variable intensity training. In most clinical settings, an intensity range of 70%-80% of peak HR determined from a symptom-limited exercise test is used. Although aerobic exercise remains the mainstay of clinical training programs, resistance training has also shown benefits, including improved muscle strength, endurance, and blood flow associated with a lower VO₂ at submaximal workloads^[65-67]. While beta blockers have numerous benefits in patients with HF, they blunt heart rate responses to exercise. It has therefore been suggested that heart rates should not be used to determine exercise capacity in these patients^[68,69]. Exercise tolerance for CHF patients may be affected by the dose changes of some medications used for CHF, and exercise prescription may need to be modified accordingly. Generally self perceived exercise workload is more practical way of determining exercise intensity than parameters like maximum heart rate^[70].

EMERGING THERAPIES

Alagebrium chloride

A thiazolium derivative, Alagebrium chloride (ALT-711) is a novel compound that breaks advanced glycation end products (AGE) crosslinks and may improve ventricular distensibility and arterial compliance. A recent prospective, open-label trial of alagebrium in elderly patients found that in clinically stable HFPEF, the 16-wk treatment with alagebrium caused regression of LVH, improved Doppler indices of diastolic function, and enhanced quality of life without altering blood pressure, arterial stiffness, or exercise tolerance^[71]. A more recent however did not support these findings^[72]. Prevention of the formation of new AGEs with exercise and breakdown of already formed AGEs with ALT may represent a therapeutic strategy for age-related ventricular and vascular stiffness^[73].

Statins

Statins have a variety of potential benefits in addition to lipid reduction that may more directly impact diastolic function. Statins may exert beneficial effects on LVH and fibrosis, and thus may directly impact HFPEF^[74]. It appears to be associated with improved survival in HFPEF^[74,75]. A study involving 270 patients with HFPEF and a follow up of 5 years demonstrated improved survival compared to patients without statin therapy (HR = 0.65; 95%CI: 0.45-0.95, $P = 0.029$)^[75]. The survival benefit was maintained after adjusting for differences in baseline characteristics, comorbidities, and other medications.

Growth differentiation factor 11

A protein belonging to the TGF- β family, growth differentiation factor 11 (GDF-11) can reverse age-related cardiac hypertrophy in mice^[76]; a finding with implications for the experimental treatment of HFPEF^[77]. Although functional benefits as measured by means of echocar-

diography were not detected after GDF-11 treatment, the results suggest that the reversal of age-related cardiac hypertrophy by pharmacologic means is potentially feasible^[76].

Gene therapy

Calcium mishandling is implicated in heart disease. Efforts are ongoing in a number of gene therapy approaches to address the calcium mishandling issue, *e.g.*, by normalising the function of calcium handling proteins such as sarcoplasmic reticulum calcium ATPase, or to introduce calcium buffers to facilitate relaxation of the heart^[78].

Parvalbumin is a calcium binding protein found in fast-twitch skeletal muscle and not normally expressed in the heart. Gene transfer of parvalbumin into normal and diseased cardiac myocytes increases relaxation rate but also markedly decreases contraction amplitude^[78]. Szatkowski *et al*^[79] have shown that parvalbumin gene transfer to the heart *in vivo* produces levels of parvalbumin characteristic of fast skeletal muscles, causes a physiologically relevant acceleration of heart relaxation performance in normal hearts, and enhances relaxation performance in an animal model of slowed cardiac muscle relaxation. They suggested that parvalbumin may offer the unique potential to correct defective relaxation in energetically compromised failing hearts because the relaxation-enhancement effect of parvalbumin arises from an ATP-independent mechanism.

NO donors

In patients with dysfunctional endothelium, the constrictor effects of catecholamines can act unopposed, which may contribute to impaired dilator responses of epicardial and resistance vessel and thereby to myocardial ischemia, which slows ventricular relaxation and increases myocardial wall stiffness. Studies have suggested that diastolic function of the heart appears to benefit from exogenous NO whereas its endogenous production does not play a major role in myocardial relaxation^[80]. Similarly, NO donors have been shown to exert a relaxant effect on the myocardium which is associated with a decrease in LV end-diastolic pressure^[80].

Ranolazine

Ranolazine is a new anti-ischemic and antianginal agent that inhibits the late sodium current, reducing the Na-dependent Ca-overload, which improves diastolic tone and oxygen handling during myocardial ischemia^[81]. In addition, ranolazine seems to exert beneficial effects on diastolic function. Most of the experimental studies performing acute exposure to ranolazine in HF report on positive effects on diastolic performance^[81]. A recent proof-of-concept study however revealed that ranolazine improved measures of hemodynamics but there was no improvement in myocardial relaxation parameters^[82].

Angiotensin receptor neprilysin inhibitor

LCZ696, a first-in-class angiotensin receptor neprilysin

inhibitor, has been assessed in patients with HFPEF in PARAMOUNT trial^[83], a phase 2, randomised, parallel-group, double-blind multicentre trial in patients with NYHA)class II-III HF, LV ejection fraction 45% or higher, and NT-proBNP greater than 400 pg/mL. In comparison with Valsartan, LCZ696 reduced NT-proBNP to a greater extent at 12. Whether these effects would translate into improved outcomes needs to be tested prospectively.

Phosphodiesterase-5 and endothelin inhibition

Despite initial encouraging results for a commonly used erectile dysfunction drug “sildenafil” to treat patients with HFPEF, the large multicentre trial “RELAX Study” failed to show any significant improvement in exercise capacity or clinical status when compared with placebo after 24 wk^[84].

Preliminary findings have suggested that cardiac endothelin-1 overexpression in a status of NO deficiency may have a role in oxidative stress, myocytes contractility, and energy metabolism^[85].

Ivabradine

Animal studies have suggested that long-term heart rate reduction induced by ivabradine may improve diastolic LV function probably involving attenuated hypoxia, reduced remodelling, and/or preserved NO bioavailability^[86]. This however is yet to be translated in human beings.

CONCLUSION

HFPEF is common and represents a major challenge in cardiovascular medicine. In contrast to advances in therapeutic options for systolic HF, there is no definitive evidence that ACE inhibitors, ARBs, beta-blockers, or aldosterone antagonists may improve outcomes in these patients. Addressing the specific aetiology and aggressive risk factor modification currently remains the mainstay in the treatment of HFPEF.

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Infiltrative cardiac lymphoma with tricuspid valve involvement in a young man

Harris Ngow Abdullah, Wan Khairina Wan Mohd Nowalid

Harris Ngow Abdullah, Department of Internal Medicine, Kulliyah of Medicine, International Islamic University Malaysia, Kuantan 25710, Malaysia

Wan Khairina Wan Mohd Nowalid, Hospital Pantai Ampang, Kuala Lumpur 57000, Malaysia

Author contributions: Ngow HA was involved in managing the patient, drafting, revising and approving the final content of the paper; Wan Mohd Nowalid WK was involved with planning and the critical revising of the content and English language of the paper.

Correspondence to: Harris Ngow Abdullah, MD, Associate Professor, Department of Internal Medicine, Kulliyah of Medicine, International Islamic University Malaysia, PO BOX 141, Kuantan 25710, Malaysia. harrisngow@gmail.com

Telephone: +609-513-2797 Fax: +609-513-3615

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large B-cell lymphoma involving the heart, in particular affecting the tricuspid valve. The clinical features in this case are clearly illustrated. The peculiarity of this case report is that besides the involvement of the right ventricle and atrium, the tricuspid valve was also infiltrated. Secondary valvular metastasis is unusual and the patient remained in remission after a course of chemotherapy.

Ngow HA, Wan Mohd Nowalid WK. Infiltrative cardiac lymphoma with tricuspid valve involvement in a young man. *World J Cardiol* 2014; 6(2): 77-80 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i2/77.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i2.77>

Abstract

Cardiac metastases are among the topics with limited systematic reviews. Theoretically, the heart can be infiltrated by any malignancy with the ability to spread to distant structures. Thus far, no specific tumors are known to have a predilection for the heart, but some do metastasize more often than others, for example, melanoma and primary mediastinal tumors. We report a case of cardiac metastasis from a diffuse large B cell lymphoma in a young man. The peculiarity of this case is that besides the involvement of right ventricle and atrium, the tricuspid valve was also infiltrated. Valvular metastasis is rarely reported in the medical literature.

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Key words: Cardiac metastases; Cardiac lymphoma; Non-Hodgkin's lymphoma; Tricuspid valve

Core tip: This manuscript describes a case of diffuse

INTRODUCTION

Primary cardiac tumors are extremely uncommon, with a reported prevalence of 0.001% to 0.28%^[1]. On the other hand, the incidence of secondary cardiac tumors is not as low as expected, ranging from 2% to 18.3%^[2]. There is growing awareness of the pathological and clinical effects of cardiac metastasis. Although metastatic heart tumors occur comparatively more frequently than primary tumors of the heart, they rarely gain clinical attention. Antemortem diagnosis of cardiac metastasis is seldom made because more than 90% are clinically silent^[1]. Besides, the signs of cardiac involvement may be overlooked when the tumors are advanced with widespread involvement of other organs. The clinical manifestations may be due to the valvular involvement or diminished cardiac function, which can be similar to a primary cardiac tumor, although intramural growth of secondary cardiac tumors is unusual. In addition, cardiac rhythm disturbances, conduction defects, syncope, distant embolism and pericardial effusion can occur. Not uncommonly, cardiac infiltration contributes to the mechanism of death in the affected person. The ability to metastasize to the heart depends

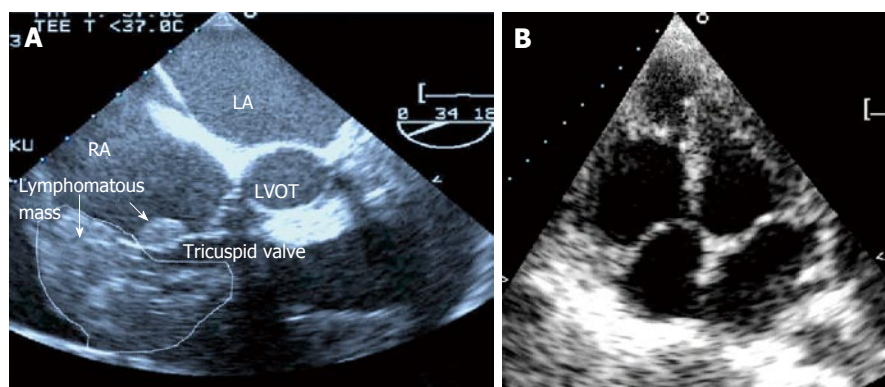


Figure 1 Transesophageal and transthoracic echocardiography before and after chemotherapy. A: Transesophageal echocardiography showed the appearance of the lymphomatous infiltration (dotted line) and a neoplastic infiltration on the tricuspid valve (short arrow). A moderate pericardial effusion is seen; B: This transthoracic echocardiography performed after 6 cycles of rituximab, cyclophosphamide, vincristine, doxorubicin and prednisolone regime showed the resolution of the tumor mass and the disappearance of the tricuspid valve tumor infiltration; RA: Right atrium; LA: Left atrium; LVOT: Left ventricular outflow tract.

on several factors, including the biological characteristics and histological subtype of the primary neoplasm, as well as the functional status of the cardiovascular system^[2,3]. Myocardial contractility may have a dual effect of both hindering and promoting the formation of cardiac metastasis; good contraction hinders the spreading of intramural tumor metastasis by facilitating lymph and blood drainage and therefore displacing any cardiac tumor-produced emboli, but on the other hand it helps neoplastic cells diffuse along the epicardial surface. Poor myocardial contractility would therefore create an opposite mechanism^[2].

CASE REPORT

A 46-year-old man was admitted with symptomatic decompensated heart failure. He complained of progressive bilateral pitting edema with dyspnea on exertion and reduced effort tolerance of 3 wk duration. However, he denied orthopnea and paroxysmal nocturnal dyspnea. He had anorexia and weight loss for the past 3 mo. He had a 5 year history of hypertension which was well-controlled with oral antihypertensives. He had multiple admissions for angina but the coronary angiogram showed no stenosis. His previous echocardiography examination was normal. He was not a smoker. There was no family history of hematogenous malignancy or exposure to radiation at a young age.

He was thin with obvious muscle wasting. His conjunctiva was pink and there was no jaundice. The jugular venous pressure was elevated and he had gross leg edema. There was multiple small shotty cervical lymphadenopathy as well as several others in the axillary and inguinal region. The apex beat was displaced but the heart sounds were normal. There was no sign of cardiac tamponade. The chest examination was insignificant. There was no hepatosplenomegaly and ascites.

The hemoglobin level was 125 g/L. The total white cell count was $8.7 \times 10^9/\text{L}$ and the platelet count was $360 \times 10^9/\text{L}$. The liver function test was normal with serum albumin of 47.1 g/L and serum globulin of 35.7 g/L. Serum transaminases were normal. The renal profile was impaired with calculated GFR of 27.5 mL/min per 1.73 m². Serum lactate dehydrogenase was 268 U/L. Other hematology parameters were within normal range.

The transthoracic echocardiogram showed global

pericardial effusion measuring 1.2-1.8 cm. The left ventricular function was 63% with mild tricuspid incompetence. An incidental finding of a right atrium and right ventricular mass was also made. An infiltrating tumor was also seen on the annulus of the anterior tricuspid valve and endocardium. The right ventricle was dilated with poor function. The right ventricle and atrium was not collapsing in systole to suggest cardiac tamponade. A repeat transesophageal echocardiography confirmed the diagnosis (Figure 1A).

Biopsy of the left axillary lymph node was performed and the histopathological examination showed diffuse malignant, large lymphoid cells consisting of centroblasts, immunoblasts and large centrocyte-like cells with occasional multinucleated cells. The large lymphoid cells were positive for CD20, weakly positive for Bcl-2 and negative for T-cell markers CD3 and CD5, and CD10. The diagnosis was diffuse large B-cell Non-Hodgkin's lymphoma (Figure 2). Computed tomography of the neck, thorax and abdomen showed multiple supra and infra-diaphragmatic enlarged lymph nodes which were suggestive of lymphoma.

The patient underwent 7 cycles of monthly chemotherapy consisting of rituximab, cyclophosphamide, vincristine, doxorubicin and prednisolone (R-CHOP). He acquired clinical remission after the chemotherapy and has remained well for the past 4 years. Clinically, there was resolution of the multiple lymphadenopathies and a repeat transthoracic and transesophageal echocardiography showed complete resolution of the tumor bulk with disappearance of the tumor infiltration on the tricuspid valve leaflet (Figure 1B). A fluorodeoxyglucose positron emission tomography (CT/FDG-PET) scan done 6 mo after the completion of chemotherapy showed no evidence of active lymphoma. The patient has remained in good health 4 years after the diagnosis and currently still under our follow up.

DISCUSSION

Cardiac metastases refer to distant spread of tumor to any structures of the cardiovascular system, including the pericardium, epicardium, myocardium, endocardium, great vessels and coronary arteries, as well as tumors affecting the heart cavities or producing intramural neoplastic thrombi. Cardiac metastases are commoner than a

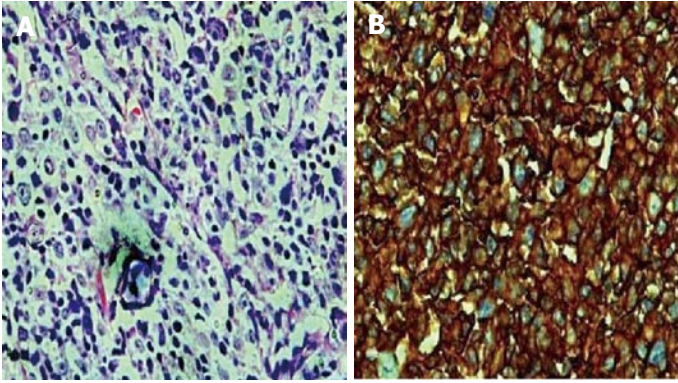


Figure 2 Histopathological staining of the left axillary lymph node.

A: The H E slide showing diffuse malignant large lymphoid cells consisting of centroblasts, immunoblasts and large centrocyte-like cells with occasional multinucleated cells (HE stain, $\times 40$); B: The tumor cells are positive for CD20 (Immunohistochemistry staining, $\times 10$).

primary tumor of the heart. The incidence from autopsy study varies between 2% and 18.3%^[1]. Common primary tumors are bronchus and breast cancers, although lymphomas, leukemia and malignant melanoma may sometimes give rise to cardiac metastasis^[4]. In a recent study from Italy by Bussani *et al.*^[2], the tumors that commonly metastasize to the heart are pleural mesothelioma (48.4%), melanoma (27.8%), lung adenocarcinoma (21%), undifferentiated carcinoma (19.5%), lung squamous cell carcinoma (18.2%), breast carcinoma (15.5%), ovarian carcinoma (10.3%), lymphoproliferative neoplasm (9.4%), bronchoalveolar carcinomas (9.8%), gastric carcinoma (8%), renal carcinomas (7.3%) and pancreatic carcinoma (6.4%)^[2]. The prevalence was somewhat different from an earlier study^[4].

Tumors can spread to the heart *via* four mechanisms, including direct tumor extension, hematogenous spread, retrograde lymphatic system dissemination and intracavitary diffusion *via* either the inferior vena cava or pulmonary vein. The different mechanisms result in specific involvement of the structure in the heart. In our patient, we postulated that the initial metastases involved the myocardium *via* one of the mechanisms above and later spread to the pericardium, resulting in moderate pericardial effusion.

Cardiac involvement in lymphoma is rare but in a recent autopsy study of malignant lymphoma, cardiac metastasis was found in 16% of the cases^[3]. Neoplastic infiltration by lymphoma typically tends to replace the myocardial tissue. Large areas of the heart are replaced by homogenous grayish white tissue with the typical “fish-meat” appearance. Despite the massive involvement of the myocardial contractile tissue, cardiac symptoms may be absent or non-specific. In the few existing cases in the medical literature, the heart seems to be more often involved in non-Hodgkin’s lymphoma, whereas the pericardium is more often metastasized by Hodgkin’s lymphoma^[5].

Clinical presentations of cardiac metastasis by lymphoma are extremely variable. They are determined by the location, size, growth rate, degree of invasiveness and friability of the neoplasm. The tumor can block the blood flow or valvular structures, leading to cardiac and valvular dysfunction. The involvement of the tricuspid valve in our patient did not give rise to severe tricuspid regurgitation and the right ventricle metastasis rather contributed

to the right heart failure. Invasion of the electrical pathways can cause arrhythmias and pericardial seeding may lead to malignant pericardial effusion or tamponade. The tumor may also cause distant embolization. In addition, sudden death may occur as a result of myocardial rupture, ventricular arrhythmias or acute myocardial infarction. Constitutional symptoms include fever, weight loss, palpitations, dyspnea and poor appetite. Lymphomatous infiltration of the heart may remain clinically silent and is often not detected until death occurs^[6].

Lymphomatous cardiac metastases are usually small and multiple, although a single large tumor mass is also observed. Focal or diffuse tumor infiltrations of the pericardium, myocardium or endocardium have been observed in lymphoma as well. In contrary to leukemic infiltration, lymphoma depositions are usually grossly discernible. The right side of the heart has been found to be more frequently involved than the left heart. This was apparent in our patient where the tumor mass was located in the right atrium and at the atrioventricular junction. Heart valves are unusual targets for tumor metastases and thus tricuspid valve involvement, as seen in our patient, is uncommon. Thus far, there is only one reported case of valvular involvement due to “neoplastic” thrombotic endocarditis by Bussani *et al.*^[7]. In our patient, the tricuspid valve involvement is most likely due to direct tumor metastasis as it completely resolved with chemotherapy, without the need for anticoagulation. Although one may argue that the valvular lesion could be due to vegetation and thrombus, they are often associated with other complications such as atrial fibrillation, ventricular aneurysm, cardiomyopathy or infective endocarditis. In the absence of these complications in our patient, the possibility of thrombosis and vegetation is less likely.

A multimodality imaging may be used to diagnose a cardiac metastasis. A plain chest radiograph lacks sensitivity and specificity as an initial diagnostic tool. Transthoracic echocardiography may be the first non-invasive screening tool but the restricted acoustic window remains a significant limitation, making transesophageal echocardiography a more sensitive technique. Computed tomography adequately demonstrates morphology, location and extent of a cardiac neoplasm with a larger view, while magnetic resonance signal intensity with contrast enhancement results in superior images identifying anatomy, blood flow, cardiac function and tissue characteriza-

tion of the mass. CT/ FDG-PET scan has recently been reported to be useful in monitoring the disease response to chemotherapy^[8,9].

Treatment options are usually aggressive chemotherapy and/or radiotherapy but the results are usually dismal. This may be due to late diagnosis or the aggressiveness of the tumor. Thus far, there is no evidence that surgical resection improves survival and furthermore it is often difficult to resect such tumors. The commonest type is B-cell lymphoma which usually responds well to chemotherapy. Chemotherapy regimens such as R-CHOP have been shown to be effective and prolong survival in the few reported cases^[10]. Without therapy, the median survival of patients is often less than 6 mo, while patients treated with chemotherapy or radiation have median survival of about 1 year. Our patient underwent 7 cycles of chemotherapy with the R-CHOP regime. The patient remained in clinical remission when he was last seen in the clinic recently.

In conclusion, cardiac involvement in lymphomatous infiltration is rare but early diagnosis is crucial in improving the prognosis. The institution of effective chemotherapy may cure the disease, avert invasive debulking surgery and maintain long term clinical remission.

COMMENTS

Case characteristics

A 46-year-old man was admitted with symptomatic decompensated heart failure.

Differential diagnosis

The peculiarity of this case is that besides the involvement of right ventricle and atrium, the tricuspid valve was also infiltrated.

Imaging diagnosis

The transthoracic echocardiogram showed global pericardial effusion measuring 1.2-1.8 cm.

Treatment

The patient underwent 7 cycles of monthly chemotherapy consisting of rituximab, cyclophosphamide, vincristine, doxorubicin and prednisolone (R-CHOP).

Related reports

Chemotherapy regimens such as R-CHOP have been shown to be effective and prolong survival in the few reported cases.

Experiences and lessons

The institution of effective chemotherapy may cure the disease, avert invasive

debulking surgery and maintain long term clinical remission.

Peer review

The manuscript describes a case of diffuse large B-cell lymphoma involving the heart, in particular affecting the tricuspid valve. Clinical features were clearly illustrated.

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|----------------------|------------|---|
| EDITORIAL | 81 | Brugada phenocopy: A new electrocardiogram phenomenon
<i>Anselm DD, Evans JM, Baranchuk A</i> |
| REVIEW | 87 | Peripartum cardiomyopathy: A puzzle closer to solution
<i>Fett JD</i> |
| MINIREVIEWS | 100 | Cardioprotection and pharmacological therapies in acute myocardial infarction: Challenges in the current era
<i>Dominguez-Rodriguez A, Abreu-Gonzalez P, Reiter RJ</i> |
| BRIEF ARTICLE | 107 | Shellfish allergy and relation to iodinated contrast media: United Kingdom survey
<i>Baig M, Farag A, Sajid J, Pothuri R, Irwin RB, Khalid HMI</i> |
| CASE REPORT | 112 | Steal syndrome secondary to coronary artery fistulae associated with giant aneurysm
<i>Vlachadis Castles A, Mogilevski T, Asrar ul Haq M</i> |

Contents

World Journal of Cardiology
Volume 6 Number 3 March 26, 2014

APPENDIX I-V Instructions to authors

ABOUT COVER Editorial Board Member of *World Journal of Cardiology*, Alberto Dominguez-Rodriguez, MD, PhD, FESC, Department of Cardiology, University Hospital of Canarias, Ofra s/n La Cuesta, La Laguna, E-38320, Tenerife, Spain

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World Journal of Cardiology
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
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Brugada phenocopy: A new electrocardiogram phenomenon

Daniel D Anselm, Jennifer M Evans, Adrian Branchuk

Daniel D Anselm, Jennifer M Evans, Adrian Branchuk, Division of Cardiology, Electrophysiology and Pacing, Queen's University, Kingston General Hospital, Kingston, Ontario K7L 2V7, Canada

Author contributions: Anselm DD and Branchuk A contributed equally to this work; Anselm DD wrote the manuscript including the initial draft and subsequent revisions; Evans JM revised the paper to meet grammatical and linguistic standards; Branchuk A designed the manuscript, contributed to revisions and served as senior advisor; all authors read and approved the final manuscript.

Correspondence to: Adrian Branchuk, MD, FACC, FRCP, Associate Professor of Medicine, Division of Cardiology, Electrophysiology and Pacing, Queen's University, Kingston General Hospital, 76 Stuart Street, Kingston, Ontario K7L 2V7, Canada. branchuk@kgh.kari.net

Telephone: +1-613-5496666 Fax: +1-613-5481387

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Abstract

Brugada phenocopies (BrP) are clinical entities that are etiologically distinct from true congenital Brugada syndrome. BrP are characterized by type 1 or type 2 Brugada electrocardiogram (ECG) patterns in precordial leads V1-V3. However, BrP are elicited by various underlying clinical conditions such as myocardial ischemia, pulmonary embolism, electrolyte abnormalities, or poor ECG filters. Upon resolution of the inciting underlying pathological condition, the BrP ECG subsequently normalizes. To date, reports have documented BrP in the context of singular clinical events. More recently, recurrent BrP has been demonstrated in the context of recurrent hypokalemia. This demonstrates clinical reproducibility, thereby advancing the concept of this new ECG phenomenon. The key to further understanding the pathophysiological mechanisms behind BrP requires experimental model validation in which these phenomena are reproduced under strictly controlled environmental conditions. The development of these validation models will help us determine whether BrP are transient alterations of sodium channels that are not repro-

ducible with a sodium channel provocative test or alternatively, a malfunction of other ion channels. In this editorial, we discuss the conceptual emergence of BrP as a new ECG phenomenon, review the progress made to date and identify opportunities for further investigation. In addition, we also encourage investigators that are currently reporting on these cases to use the term BrP in order to facilitate literature searches and to help establish this emerging concept.

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Key words: Brugada phenocopy; Brugada syndrome; Electrolytes; Myocardial ischemia; Pulmonary embolism; Cardiomyopathy; Electrocardiogram filters

Core tip: Diagnostic distinctions between Brugada phenocopies (BrP) and Brugada syndrome (BrS) are: (1) BrP patients have a reversible underlying condition and upon resolution of this condition, the electrocardiogram normalizes; (2) BrP patients have a low pretest probability of BrS as opposed to a high pretest probability in patients with true congenital BrS; and (3) BrP patients have a negative sodium channel blocker test, while patients with BrS have a positive test. The different electrocardiographic response to the provocative challenge highlights a pathophysiological divergence when comparing BrP and BrS. This suggests alternative underlying mechanisms with various genetic, structural and environmental interactions yet to be elucidated.

Anselm DD, Evans JM, Branchuk A. Brugada phenocopy: A new electrocardiogram phenomenon. *World J Cardiol* 2014; 6(3): 81-86 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i3/81.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i3.81>

INTRODUCTION

Brugada syndrome (BrS) is a congenitally inherited car-

diac channelopathy characterized by type 1 and type 2 electrocardiogram (ECG) patterns in leads V1-V3 that predisposes individuals to malignant ventricular arrhythmias and sudden cardiac death^[1]. Brugada phenocopies (BrP) are clinical entities that have ECG patterns that are identical to true congenital BrS but are elicited by various other factors, such as myocardial ischemia, metabolic abnormalities, mechanical mediastinal compression and poor ECG filters^[2,3]. In this editorial, we discuss the conceptual emergence of BrP as a new ECG phenomenon, review the progress made to date and identify opportunities for further investigation.

THE BRUGADA ECG PATTERN

True congenital BrS is characterized by two ECG patterns in leads V1-V3: The typical type 1 “coved” or the type 2 “saddleback” patterns. The type 1 pattern has a high take-off ST-segment elevation that is ≥ 2 mm followed by a down-sloping concave or rectilinear ST-segment with a negative symmetric T-wave (Figure 1A)^[1]. The type 2 pattern is defined as a high take-off (r') that is ≥ 2 mm from the isoelectric baseline, followed by ST-segment elevation that is convex with respect to the isoelectric baseline with elevation ≥ 0.05 mV, with variable T-wave in lead V1 and positive or flat T-wave in lead V2 (Figure 2A)^[1].

THE BRUGADA PHENOCOPY

BrP are clinical entities that are etiologically distinct from true congenital BrS. BrP are defined by ECG patterns that are identical to BrS but are elicited by various clinical circumstances. The term phenocopy was chosen because it was previously used to describe an environmental condition that imitates one produced by a gene; therefore, it served as a reasonable and succinct description for all acquired Brugada-like ECG manifestations^[4].

Since the initial reports, type 1 BrP have been reported in the context of an acute inferior ST-segment elevation myocardial infarction with right ventricular involvement (Figure 1B)^[5]; concurrent hyperkalemia, hyponatremia and acidosis (Figure 1C)^[6,7]; acute pulmonary embolism (Figure 1D)^[8,9]; and hypokalemia in the context of congenital hypokalemic periodic paralysis (Figure 1E)^[10,11]. Similarly, type 2 BrP have been reported immediately post-electrocution accidental injury (Figure 2B)^[12], in the context of congenital pectus excavatum causing mechanical mediastinal compression (Figure 2C)^[13]; and as a result of an inappropriate high-pass ECG filter (Figure 3)^[14].

In each of these prior reports, the BrP was observed in the context of a singular inciting clinical event such as myocardial ischemia or metabolic derangement. Finally, the BrP concept was advanced by demonstrating clinical reproducibility in the context of recurrent hypokalemia^[15]. Briefly, a young patient with diarrhea was admitted to hospital due to severe hypokalemia (K 1.5 mEq/L) with concurrent acidosis. The ECG depicted a typical type 1 Brugada ECG pattern (Figure 3A). Upon correc-

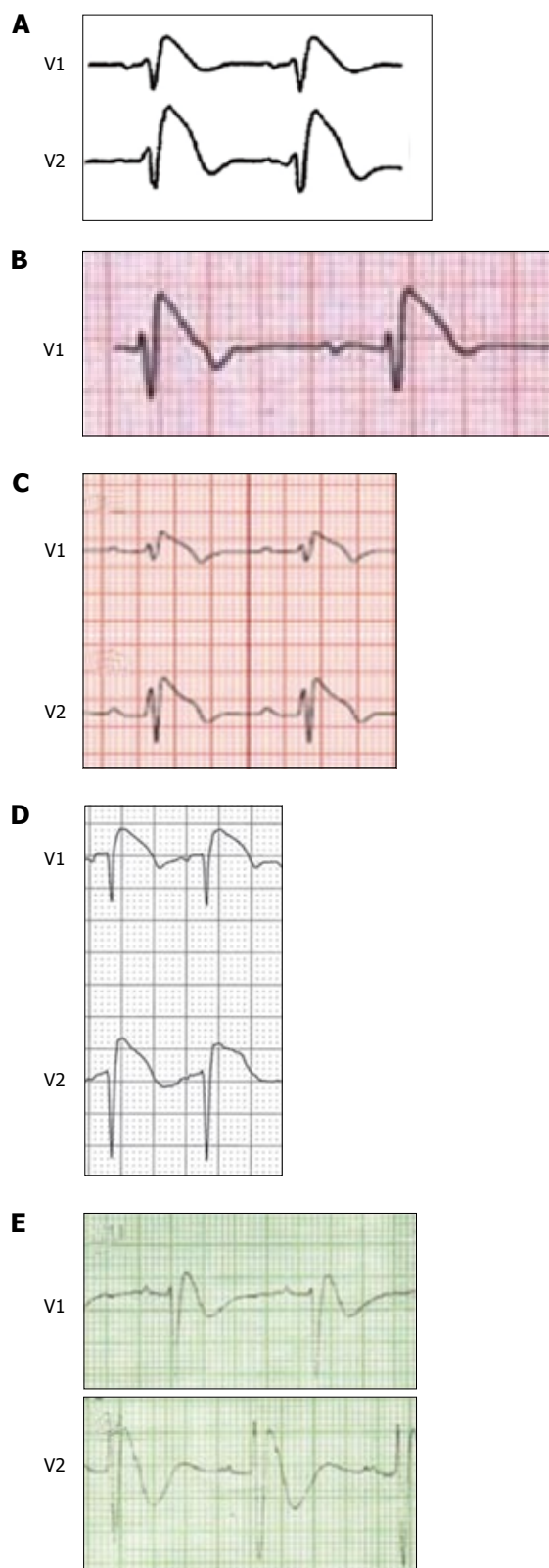


Figure 1 Type 1 Brugada phenocopies. A: True congenital type 1 Brugada electrocardiogram (ECG) pattern; B: Type 1 Brugada phenocopies (BrP) in a patient with an acute inferior ST-segment elevation myocardial infarction with right ventricular involvement; C: Type 1 BrP in a patient with concurrent hyperkalemia, hyponatremia and acidosis; D: Type 1 BrP in a patient with an acute pulmonary embolism; E: Type 1 BrP in a patient with hypokalemia in the context of congenital hypokalemic periodic paralysis.

tion of the metabolic abnormalities, the ECG promptly

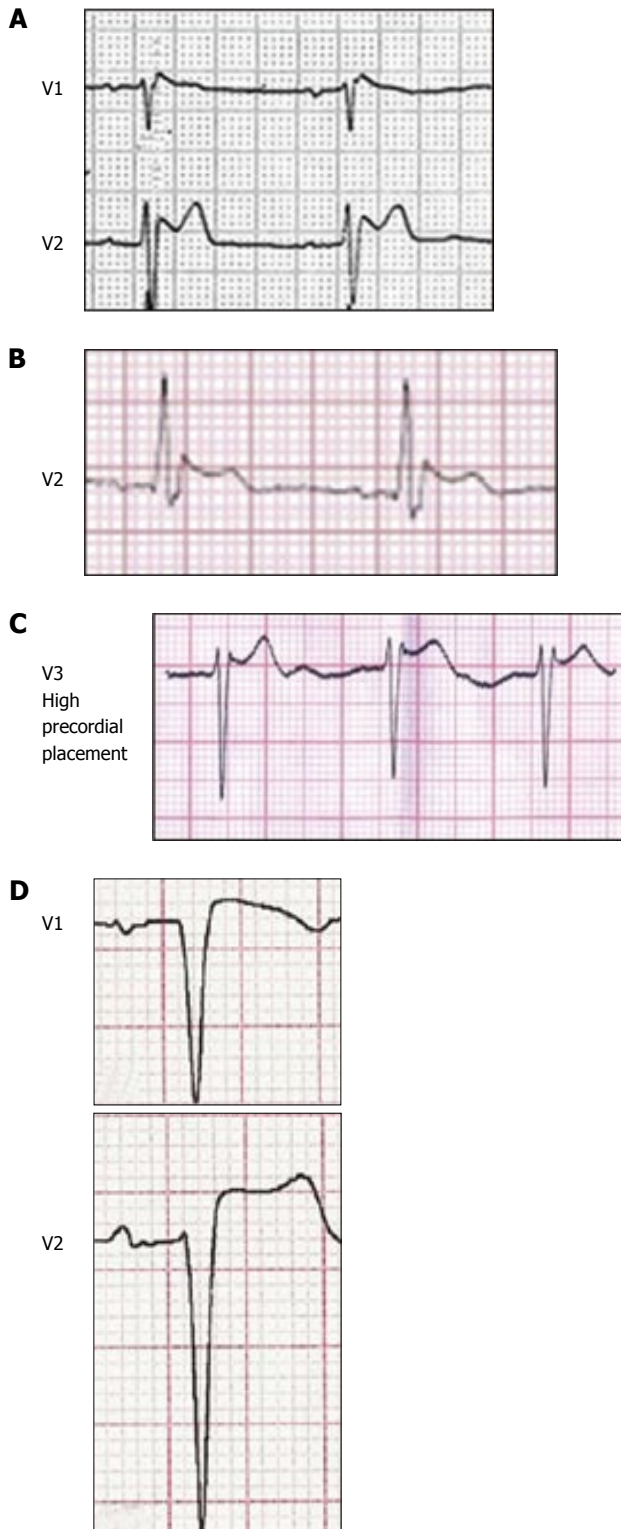


Figure 2 Type 2 Brugada phenocopies. A: True congenital type 2 Brugada electrocardiogram (ECG) pattern; B: Type 2 Brugada phenocopies (BrP) in a patient with an accidental electrocution injury; C: Type 2 BrP in a patient with congenital pectus excavatum causing mechanical mediastinal compression; D: Type 2 BrP as a result of an inappropriate high pass ECG filter.

returned to normal (Figure 3B). A subsequent flecainide provocative challenge did not induce a type 1 Brugada ECG pattern, thereby excluding myocardial sodium channel dysfunction. During the same hospitalization period, the patient experienced ongoing diarrhea with a sec-

Table 1 Brugada phenocopy etiological categories

Etiological category
Metabolic conditions
Mechanical compression
Ischemia and pulmonary embolism
Myocardial and pericardial disease
ECG modulation
Miscellaneous

Reproduced with permission^[9]. ECG: Electrocardiogram.

ond episode of hypokalemia (K 2.6 mEq/L); however, without concurrent acidosis. The ECG again depicted a typical type 1 Brugada ECG pattern (Figure 3C) which resolved after correction of the metabolic abnormality (Figure 3D). This case report is important because it was the first to demonstrate clinical reproducibility of the BrP.

DIFFERENTIATING BRUGADA PHENOCOPY FROM BrS

Currently, a total of 55 case reports, editorials, letters, abstracts and book chapters have been published that discuss the etiology, pathophysiology and conceptual evolution of BrP^[16]. This has led to the current BrP etiological categories (Table 1) and diagnostic criteria (Table 2).

The diagnostic distinction between BrP and true congenital BrS focuses on a few key features. First, patients with BrP have a reversible underlying condition such as adrenal insufficiency, hypokalemia or myocardial ischemia that elicits or induces the Brugada ECG pattern. Once this underlying condition resolves there is prompt normalization of the ECG. This is contrary to true congenital BrS where the ECG manifestations are unmasked by sodium channel blockers, vagotonic agents, febrile states and various metabolic conditions. Second, patients with BrP have a low clinical pretest probability of true congenital BrS as opposed to a high clinical pretest probability in patients with true congenital BrS who have a documented personal history of cardiac arrest, nonvagal syncope or a family history of sudden cardiac death^[1]. Third, patients with BrP have a negative provocative challenge with a sodium channel blocker, while those with true congenital BrS have a positive provocative challenge (Table 2).

The different response to a sodium channel provocative challenge highlights a fundamental pathophysiological divergence when comparing BrP and BrS patients who are exposed to similar environmental stimulus. For example, Postema *et al.*^[17] reported a case of true congenital BrS in the context of hyperkalemia and acidosis. This patient presented with a type 1 Brugada ECG pattern and underwent a positive provocative challenge with ajmaline and negative sodium channel voltage-gated type V α subunit genetic testing. Interestingly, Recasens *et al.*^[6] and Anselm *et al.*^[7] reported a similar case where the patient presented with a type 1 Brugada ECG pattern in the context of hyperkalemia, hyponatremia and acidosis.

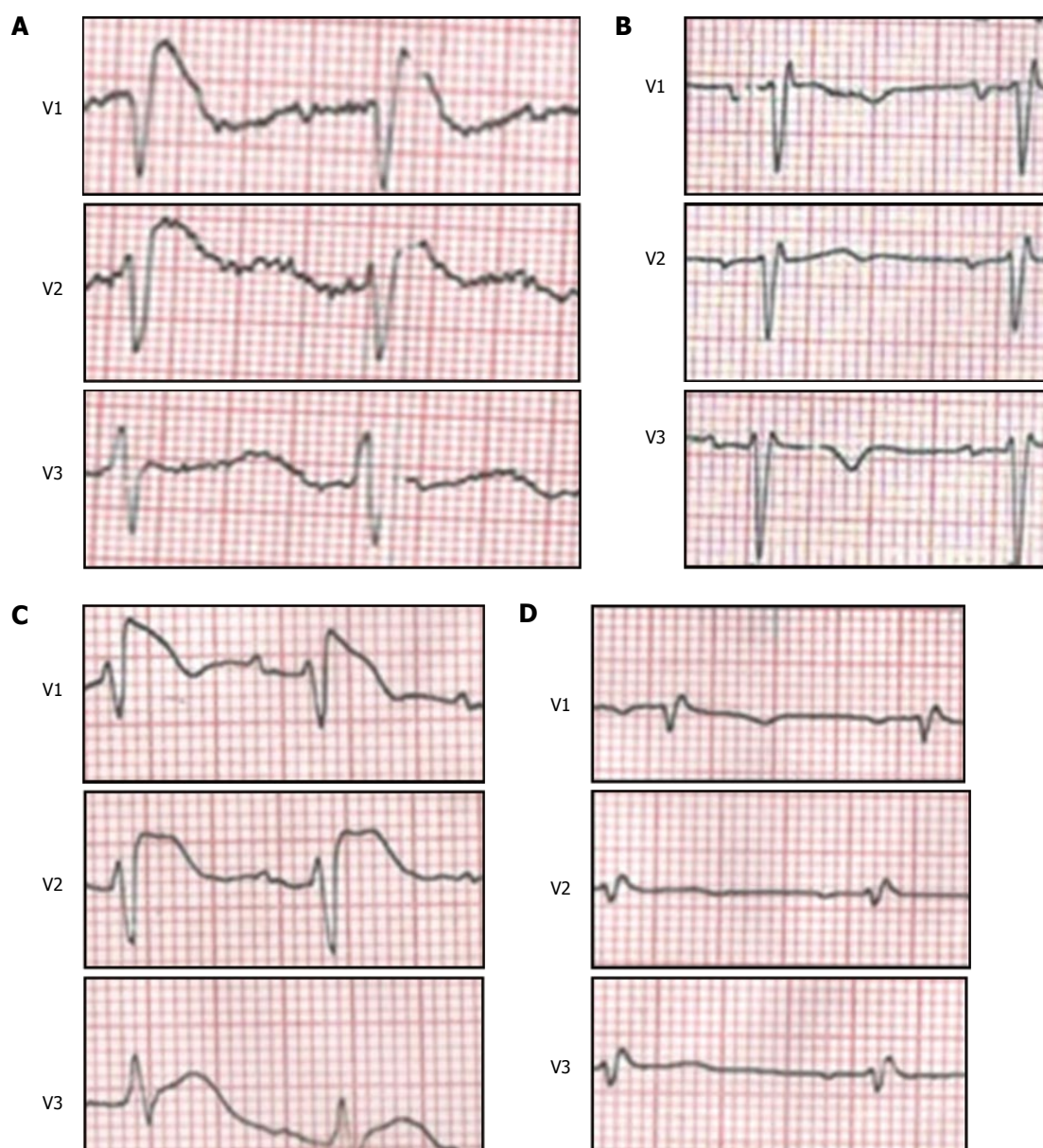


Figure 3 Brugada phenocopy clinical reproducibility. A: Electrocardiogram (ECG) on presentation while the patient is hypokalemic consistent with a type 1 Brugada ECG pattern; B: After correction of the electrolyte abnormality, the ECG normalizes; C: While in hospital, the patient again becomes hypokalemic with recurrence of the type 1 Brugada ECG pattern; D: Subsequent normalization of the ECG pattern after potassium is corrected.

This patient had a negative flecainide provocative challenge. Given the negative sodium channel provocative test, this suggests alternative underlying mechanisms with various genetic, structural and environmental interactions that are yet to be elucidated^[18].

Additionally, while patients with high-risk true congenital BrS are candidates for cardioverter-defibrillator implantation, the clinical implications of patients with BrP remain unknown. Therefore, BrP treatment recommendations at this time would suggest focusing on the resolution of the underlying condition as further intervention has not yet been investigated or validated.

BRUGADA PHENOCOPY: FUTURE DIRECTIONS

The chronological emergence of new ECG phenomena

should include: (1) phenomenological observation; (2) speculation on pathophysiological mechanisms; (3) clinical reproducibility; and (4) experimental model validation. The literature to date has demonstrated steps (1), (2) and (3); however, the key to further understanding the mechanisms behind BrP requires that these phenomena be reproduced under strictly controlled environmental conditions^[19-21]. The development of experimental validation models will help us determine whether BrP are transient alterations of the sodium channels that cannot be reproduced with a provocative sodium channel blocking test, or if they are a malfunction of other myocardial ion channels. Similarly, exposing a genetic model of true congenital BrS to the common conditions that elicit BrP would aid in understanding whether BrP and BrS are entities that belong to the same spectrum of disease, or are completely different entities. In that sense, the model that

Table 2 Criteria to differentiate the Brugada electrocardiogram pattern, Brugada phenocopy and true congenital Brugada syndrome

Brugada ECG pattern
The ECG pattern has a type 1 or type 2 Brugada morphology as currently defined by Bayés de Luna <i>et al</i> ^[1]
Diagnostic criteria for BrP
The ECG pattern has a type 1 or type 2 Brugada morphology
The patient has an underlying condition that is identifiable
The ECG pattern resolves after resolution of the underlying condition
There is a low clinical pretest probability of true BrS determined by lack of symptoms, medical history and family history
Negative provocative testing with sodium channel blockers such as ajmaline, flecainide or procainamide
Provocative testing not mandatory if surgical RVOT manipulation has occurred within the last 96 h
The results of genetic testing are negative (desirable but not mandatory because the SCN5A mutation is identified in only 20% to 30% of probands affected by true BrS)
Features that suggest true congenital BrS
The ECG pattern has a type 1 or type 2 Brugada morphology
There is a high clinical pretest probability of true congenital BrS determined by presence of symptoms, medical history and family history
Positive provocative testing with sodium channel blockers such as ajmaline, flecainide or procainamide. This indicates sodium channel dysfunction consistent with true BrS
Genetic testing is positive in about 20% to 30% of probands

Reproduced with permission^[16]. RVOT: Right ventricular outflow tract; SCN5A: Sodium channel voltage-gated type V alpha subunit; BrP: Brugada phenocopies; BrS: Brugada syndrome.

discovered genetic alterations in patients with acquired long QT^[22] should serve as inspiration to develop the BrP experimental model.

In order to learn about the natural history of BrP, an international online database that allows for longitudinal follow-up is in development at www.brugadaphenocopy.com. We encourage all investigators that are currently reporting on these cases to use the term Brugada phenocopy in order to facilitate literature searches and to help establish this emerging concept^[23,24].

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Peripartum cardiomyopathy: A puzzle closer to solution

James D Fett

James D Fett, Investigations of Pregnancy Associated Cardiomyopathy (IPAC), Peripartum Cardiomyopathy Network of North America (PCN), Cardiovascular Institute, University of Pittsburgh Medical Center, Pittsburgh, PA 15213, United States
James D Fett, Department of Adult Medicine, Hospital Albert Schweitzer, Deschanelles, Haiti

Author contributions: Fett JD solely contributed to this paper.
Correspondence to: James D Fett, MD, Department of Adult Medicine, Hospital Albert Schweitzer, Deschanelles, Haiti, c/o 2331 Mt. Hood Ct. SE, Lacey, WA 98503, United States. fett.sprunger@comcast.net
Telephone: +1-360-4385270

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Core tip: The purpose of this review is to highlight the important advances that have brought us nearer to the solution of this puzzle, focusing on what we have learned about peripartum cardiomyopathy (PPCM) since 2000; and what still remains unanswered. There have been many improvements in outcome. Increased understanding of the pathogenesis of PPCM is detailed herein; however, we still do not know the actual triggers that initiate the pathological process; but realize that cardiac angiogenic imbalances resulting from complex pregnancy-related immune system and hormonal changes play a key role.

Abstract

Peripartum cardiomyopathy (PPCM) represents new heart failure in a previously heart-healthy peripartum patient. It is necessary to rule out all other known causes of heart failure before accepting a diagnosis of PPCM. The modern era for PPCM in the United States and beyond began with the report of the National Institutes of Health PPCM Workshop in 2000, clarifying all then-currently known aspects of the disease. Since then, hundreds of publications have appeared, an indication of how devastating this disease can be to young mothers and their families and the urgent desire to find solutions for its cause and better treatment. The purpose of this review is to highlight the important advances that have brought us nearer to the solution of this puzzle, focusing on what we have learned about PPCM since 2000; and what still remains unanswered. Despite many improvements in outcome, we still do not know the actual triggers that initiate the pathological process; but realize that cardiac angiogenic imbalances resulting from complex pregnancy-related immune system and hormonal changes play a key role.

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Fett JD. Peripartum cardiomyopathy: A puzzle closer to solution. *World J Cardiol* 2014; 6(3): 87-99 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i3/87.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i3.87>

INTRODUCTION

Peripartum cardiomyopathy (PPCM) represents new heart failure in a previously heart-healthy peripartum patient^[1]. It is necessary to rule out all other known causes of heart failure before accepting a diagnosis of PPCM. Specific echocardiographic criteria define the requirement of systolic heart dysfunction with a left ventricular ejection fraction (LVEF) less than 0.45^[2]. Even if the heart failure has its onset slightly out of the historic definition of time range from one month before delivery to 5 months postpartum, the process is similar, designated as pregnancy-associated cardiomyopathy^[3].

The modern era for PPCM in the United States began with the report of the NIH PPCM Workshop Group^[1] in 2000, describing currently known aspects of the disease; including definition, incidence, potential etiologies, risk factors, diagnosis and management. Since then hundreds

Table 1 Summary of current state of knowledge about peripartum cardiomyopathy

What do we know about PPCM?	What remains unknown about PPCM?
Awareness is important for making an earlier diagnosis with less dysfunction	Actual “triggers” that initiate the process
Hypertension in pregnancy increases risk for development of PPCM	Role of virus in pathogenesis
Most serious complications can be decreased or avoided	Why higher incidence and more severe disease in those with African heritage
Full recovery occurs more frequently than with any other cardiomyopathy	How important role cardiac autoantibodies play in pathogenesis
Autoimmunity (or immune system dysfunction) a part of pathogenesis	The extent and details of genetic factors
Inflammatory cardiomyopathy is common	Importance of the role of prolactin and prolactin inhibition treatment
Higher incidence and more severe disease in those of African heritage	Importance of the role of sFLT1 in pathogenesis
There can be a genetic predisposition	Why do some recovered have a relapse of heart failure with subsequent pregnancy
Effective evidence-based treatment guidelines available	Role of micronutrients and trace metals in pathogenesis
Most recovered do not have a relapse of heart failure in subsequent pregnancy	
Occurs globally, but with geographic variations for incidence and unique characteristics	

PPCM: Peripartum cardiomyopathy.

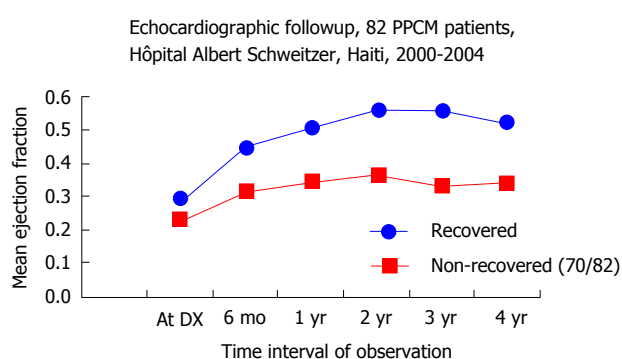


Figure 1 Lower systolic heart function at diagnosis of peripartum cardiomyopathy often means less recovery, “start low, stay low”^[6,14,26]. PPCM: Peripartum cardiomyopathy.

of publications have appeared, an indication of the pressing nature of the disease and the desire to find solutions for its cause and better treatment. There have been numerous excellent recent reviews^[4-8], so this review is not designed to cover the broad basic facets of PPCM. Instead, the purpose of this review is to highlight the important advances that have brought us nearer to the solution of this puzzle; and to identify those key areas that remain without definitive answers. The summarized points of emphasis are listed in Table 1, and discussed individually below.

INCREASING AWARENESS OF PPCM

We know that it helps to have a high index of suspicion that pregnancy-associated heart failure could occur in a previously heart-healthy young woman. Although it is possible that a fulminant myocarditis/cardiomyopathy can suddenly appear without prior warning and awareness, almost all of these women, upon reflection, can recognize that they experienced signs and symptoms earlier by days and weeks. My incessant theme is this: Physicians, nurses and patients must be alert to the possibility that a young woman, despite the lack of any type of heart

problem in her medical history, may develop a serious cardiomyopathy with acute onset of heart failure in the setting of pregnancy^[9].

One reason for the importance of this heightened awareness is that if the patient and her health care providers know about PPCM there is greater potential to recognize it earlier. An earlier detection means that the baseline or diagnostic echocardiographic LVEF is likely to be higher; and when it is in the range of 0.35 or above, the chances for full recovery are much greater (Table 2)^[10-17]. At that level the mortality rate is essentially zero and the full recovery rate approaches 100%. When at-diagnosis LVEF is lower, rate of progression towards recovery is slower, particularly in those of African heritage (See Figure 1 and Table 2).

Studies have shown that lower at-diagnosis LVEF is found when there are delays in diagnosis. This is well demonstrated in the study by Goland *et al*^[10] of 182 United States PPCM patients. They looked at major adverse events, defined as either death or complications that were life threatening. “Delay in diagnosis” referred to patient estimate of time from onset of symptoms to time of confirming the diagnosis of PPCM. 136 PPCM patients who had no adverse events had a mean delay in diagnosis of 1.7 wk while 46 PPCM patients who did have major adverse events had a mean delay in diagnosis of 3.8 wk ($P = 0.02$). Time-of-diagnosis LVEF for those without serious adverse events showed mean value of 0.31, while those with the same serious adverse events showed mean of 0.24 ($P < 0.001$).

HYPERTENSION IN PREGNANCY POSES HIGHER RISK FOR DEVELOPMENT OF PPCM

Up to one-half of PPCM patients have experienced some form of hypertension during their index PPCM pregnancy^[4,5]. Recent clues about the importance of hypertension in pregnancy derive from studies of toxemia of pregnan-

Table 2 Echocardiographic parameters at diagnosis as predictors of recovery (Left ventricular ejection fraction $\geq 50\%$) for peripartum cardiomyopathy *n* (%)

Study	Recovered	Non-recovered	P
Goland <i>et al</i> ^[10,11] (25% African-American)	115 (61.5)	72 (38.5)	
Diagnosis mean LVEF	0.31	0.23	< 0.0001 ^a
Diagnosis mean LVEDd (cm)	5.5	6.1	0.002 ^a
Amos <i>et al</i> ^[12] (51% African-American)	22 (44.9)	27 (55.1)	
Diagnosis mean LVEF	0.23	0.20	0.16
Mean LVEF (%) at 2 mo	43	24	< 0.001 ^a
Diagnosis mean LVEDd (cm)	5.6	6.2	0.01 ^a
Modi <i>et al</i> ^[13] (88.6% African-American)	14 (35)	26 (65)	
Diagnosis mean LVEF	0.29	0.21	0.02 ^a
Diagnosis mean LVEDd (cm)	5.9	6.2	0.16
Fett <i>et al</i> ^[14] (all African heritage)	32 (27.6)	84 (72.4)	
Diagnosis mean LVEF	0.28	0.23	0.002 ^a
Diagnosis mean LVEDd (cm)	5.6	5.9	0.03 ^a
Safirstein <i>et al</i> ^[15] (3.6% African-American)	43 (78.2)	12 (21.8)	
Diagnosis mean LVEF	0.29	0.24	0.13
Diagnosis mean LVEDd (cm)	5.4	5.9	0.21
Diagnostic LVEF > 0.35	25/25	0	< 0.001 ^a
¹ Haghikia <i>et al</i> ^[16]	45 (47)	51 (53)	
Diagnosis mean LVEF	0.28	0.17	< 0.0001 ^a
McNamara <i>et al</i> ^[17] (30% African-American)	59 (65)	32 (35)	
Diagnostic LVEF < 0.30	10/30 (33)	20/30 (67)	0.001 ^a
Diagnostic LVEF ≥ 0.30	58/70 (82.9) ²	21/70 (17.1) ²	0.001 ^a

¹For this group, recovery defined as LVEF 0.55, mean LVEF shown for improved *vs* non-improved; ²Pending last echo late data entry from 12 mo postpartum. LVEDd: Left ventricular end-diastolic diameter; LVEF: Left ventricular ejection fraction; Recovered: Last LVEF ≥ 0.50 ; Non-recovered: Last LVEF < 0.50;

^aP ≤ 0.05 *vs* non-recovered.

cy (eclampsia and preeclampsia), showing the importance of some biomarkers that assist in early identification of patients at high risk^[18-20]. These same biomarkers appear to be also present in PPCM not only as markers, but strongly suspect as causal factors in the pathogenesis of PPCM^[21]. The functional cardiac abnormalities in severe preeclampsia reflect a diastolic dysfunction, and some of these women also go on to classical systolic dysfunction heart failure that meet diagnostic criteria for PPCM^[22,23].

A recent epidemiology report out of North Carolina^[24] shows that out of 79 PPCM patients, 51 (65%) had some form of hypertension. Eleven, (13.9%) had preeclampsia, 18 (22.8%) had gestational hypertension, 10 (12.7%) had chronic hypertension, 10 (12.7%) had chronic hypertension and preeclampsia, 1 had eclampsia. Only one had hemolysis, elevated liver enzymes and low platelet count syndrome.

PREVENTING SERIOUS COMPLICATIONS OF PPCM

Most serious complications of PPCM can be either avoided or decreased (See Case Reports 1 through 5). The most serious complications of PPCM (ventricular tachyarrhythmias, thromboembolic events, chronic cardiomyopathy) are found when the diagnostic or baseline LVEF is below 0.30 to 0.35^[3-5,9-17]. In the Investigations of Pregnancy Associated Cardiomyopathy (IPAC) study, 5/6 major adverse events (death or transplant or left ventricular assist device) occurred in those with baseline LVEF < 0.30, confirming that women with severe systolic dysfunction at presentation have the poorest out-

comes^[17]. As such, this group may represent a target for future interventional trials.

It is also important to be certain that the best treatment is being implemented for all; but particularly for those in this LVEF under 0.30 category so as to help prevent the major complications: Adequate anticoagulation to help prevent thromboembolic phenomena; heart rhythm monitoring and devices to recognize and treat dangerous arrhythmias; and full use of evidence-based AHA Guideline therapy to help achieve eventual recovery^[25].

REMARKABLE RECOVERY POTENTIAL

Full recovery of heart function occurs more frequently in PPCM than with any other dilated cardiomyopathy. Even with the very limited resources in Haiti, an organized program to diagnose and manage PPCM, with the first population-based PPCM registry, demonstrated the ability to improve full recovery from less than 4% to over one-third of women over a period of 4 years^[26]. The first United States prospective study of PPCM, the IPAC study showed that full recovery (LVEF ≥ 0.50) at 6 mo postpartum came to a remarkable over 65 % of patients^[17]. It is important to note that this level of full recovery occurred without the use of bromocriptine inhibition of the lactating hormone, prolactin. This is discussed in greater detail later. Other studies, all retrospective in nature, have also confirmed high rates of recovery^[11,12,27].

Table 2 confirms the importance of diagnostic levels of systolic heart function (LVEF) to recovery. Health care providers and women in the latter stages of pregnancy are becoming more aware of the importance of

early identification of PPCM; and are becoming more alert about how to differentiate normal late pregnancy signs and symptoms from early heart failure symptoms^[9].

IMMUNE SYSTEM CHANGES IN PATHOGENESIS OF PPCM

Immune system changes (autoimmunity or immune system dysfunction) are an important part of the pathogenesis of PPCM^[28]. Alterations in cellular immunity have been observed in PPCM patients compared to normal postpartum women. An increase in the activation of regulatory T-cells and innate immunity is a necessary part of all pregnancies. However, there is an increase of T cells (CD3⁺ CD4⁺ CD8⁺ CD38) in PPCM patients compared to healthy postpartum patients. Natural killer (NK) cells (CD3⁺ CD56⁺ CD16⁺) are significantly reduced in PPCM patients compared to healthy postpartum women. Furthermore, while the decrease in percent of NK cells is similar in both black and white PPCM patients at entry to the study, this decrease persisted 2 mo later only in blacks^[29-31]. IPAC, with a prospective study of 100 North American PPCM patients, is currently investigating if this immune system activation correlates with recovery outcomes^[17]. (IPAC available at <http://www.peripartumcmnetwork.pitt.edu>). The earlier Investigations of Myocarditis and Acute Cardiomyopathy studies identified comparable findings in their PPCM cohort^[30]. This remarkable finding relating to differences between African heritage and Caucasian PPCM mothers with respect to NK cells is undergoing additional studies^[31].

INFLAMMATORY CARDIOMYOPATHY IN PATHOGENESIS OF PPCM

A cardiomyopathy with inflammatory cytokines is common in PPCM. This inflammatory process may be either cellular or molecular non-cellular or both^[27,32-34]. Mean serum levels of high sensitivity C-Reactive Protein (hsCRP), a simple and inexpensive laboratory estimate reflecting proinflammatory cytokines, were found to be significantly elevated in 22 Haitian PPCM patients compared to 14 non-PPCM Haitian mothers (144.3 mg/L, range 2.8-946 *vs* 5.2 mg/L, range 1.8-9.9, *P* < 0.001)^[14]. In the same population, significantly higher mean serum hsCRP levels were found in recovered PPCM patients compared to non-recovered PPCM patients (417 mg/L compared with 27 mg/L, *P* = 0.004), suggesting that a vigorous inflammatory response favored chances of recovery^[33,34]. Elevated mean serum hsCRP levels have also recently been reported in 52 Chinese PPCM patients compared to 52 non-PPCM controls (28.2 mg/L *vs* 6.2 mg/L, *P* < 0.05)^[35]. In South African PPCM patients at diagnosis, higher levels of serum hsCRP correlated with left ventricular end diastolic diameter (*P* = 0.003) and inversely with LVEF (*P* = 0.015)^[32]. A recent article describing a prospectively identified cohort of 46 PPCM patients in India also reports significantly elevated levels

of serum hsCRP, Tumor Necrosis Factor- α , and Interleukin-6^[36]. These inflammatory markers also helped to predict outcome.

The biomarker of serum hsCRP will only be elevated in the presence of an inflammatory cardiomyopathy, a frequent occurrence in PPCM. However, one would not expect an elevation of serum hsCRP if no inflammatory cardiomyopathy exists, such as in the presence of a familial dilated cardiomyopathy or in a relapse of heart failure in a previously unrecovered PPCM mother in a post-PPCM pregnancy.

Multiple proinflammatory cytokines involved in the pathogenesis of PPCM include Fas, hsCRP, Interferon- γ , Interleukin-6, Transforming Growth Factor- β , Tumor Necrosis Factor- α and others in the process of evaluation^[28,34,37].

GENETIC FACTORS IN PPCM

An important proportion of PPCM patients, around 5%-10%, have either a genetically caused condition (which would make the correct diagnosis familial dilated cardiomyopathy) or a genetic predisposition to develop PPCM when linked with additional factors^[5,38]. Higher incidence of PPCM in those of African origin can be attributed in part to genetic factors, although environmental factors may also play an important role^[39,40]. A genome-wide association of PPCM with chromosome 12p11 locus has been reported by Horne *et al*^[38]. There may also be a genetic predisposition to the development of PPCM, with another factor or factors, involving a complex interaction of pregnancy-associated immune system changes^[41].

It is important to explore further the relationship of PPCM with Idiopathic dilated cardiomyopathy (IDCM) since clinically there are many similarities. Up to one-quarter of familial dilated cardiomyopathy patients and 18% of sporadic IDCM have the presence of TTN, the protein encoding the sarcomere protein titin^[42]. What proportion of PPCM patients also have this gene? Additional studies need to be carried out exploring the finding of a single nucleotide polymorphism, rs258415, to have genome-wide significance in PPCM versus control mothers^[38]. Additional studies are ongoing and will certainly continue to add to our knowledge about inherited patterns and genetic influences in PPCM.

EVIDENCE-BASED TREATMENT OF PPCM

There is effective evidence-based treatment available with the combination of tolerable dosages of diuretics, Angiotensin Converting Enzyme Inhibitors (ACEI) and beta-blockers (BB) as outlined in published Guidelines. There need be no guess work in the application of effective treatment for PPCM since proved effective treatment of heart failure from PPCM is available and clearly defined in the American Heart Association and European Society of Cardiology Guidelines for treatment of heart failure with reduced LVEF^[25,43]. This evidence-based treatment (categories of Class I: "Benefit exceeds risk, should use" and Level of Evidence A: "Data from multiple clinical

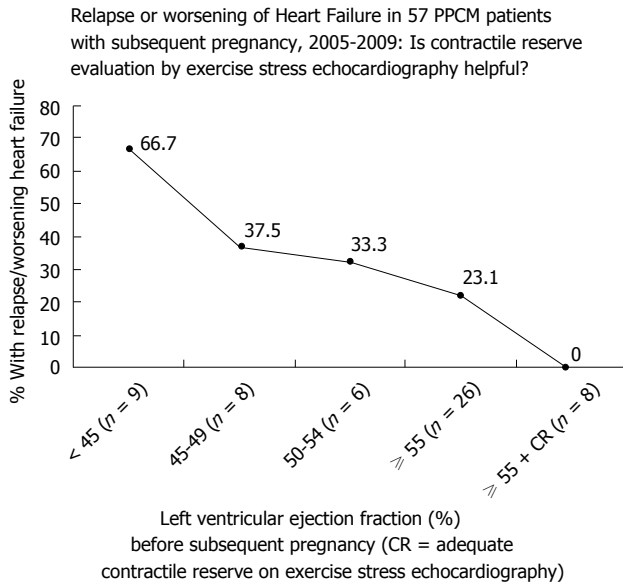


Figure 2 Risk for relapse of heart failure in a post-peripartum cardiomyopathy pregnancy^[53,54]. PPCM: Peripartum cardiomyopathy.

trials and multiple populations”) for systolic heart failure with decreased LVEF consists in giving tolerable dosages of diuretics, ACEI (replaced by hydralazine with or without nitrates if still pregnant or breastfeeding) and BB. Angiotensin receptor blockers (ARB) may be used if there is ACEI intolerance; but just as with ACEI, they are not safe to take during pregnancy or conception. Otherwise, this Guideline-recommended treatment is the same as for heart failure in other non-ischemic cardiomyopathies.

Very severe systolic dysfunction at diagnosis with circulatory collapse will require other treatment for hemodynamic support; and prevent the initial use of BB. As mentioned in the section on thromboembolic events, appropriate anticoagulation until improvement of LVEF above 0.30-0.35 is indicated.

Work by Hilfiker-Kleiner *et al*^[44] and Sliwa *et al*^[45] with respect to potential cardiotoxic prolactin metabolites has stimulated interest in the use of prolactin inhibition by bromocriptine. In regards to the use of bromocriptine, the recent study out of Germany^[16], found the greatest improvement (55 out of 57 or 96%) occurred in PPCM patients receiving combination treatment of BB, ACEI/ARB and bromocriptine (2.5-5 mg/d for 4 wk). These investigators reported “full recovery” (LVEF ≥ 0.55) for 45 out of 96 (47%) PPCM patients; but that there was no statistically significant difference in those who reached full recovery for the 64 who received bromocriptine compared with the 32 who did not receive bromocriptine. Out of 96 PPCM patients, 14 failed to improve. All of these had baseline LVEF ≤ 0.25 .

These European investigators indicate that bromocriptine “may not be sufficiently effective in all patients, especially in PPCM patients with very low baseline EF”^[16]. Their cohort of PPCM patients with very low baseline EF also frequently could not receive BB treatment due to low blood pressure and bradycardia. It is to

be noted that the full recovery rates for these European patients were very similar to those reported by North American IPAC investigators, a study in which bromocriptine had not been a part of the treatment^[17].

The best tolerated dosages of combination BB and ACEI treatment will be the most helpful in moving towards full recovery. A serious deficiency in treatment would be the use of only BB or only ACEI/ARB instead of a combination of the two at tolerated dosages. Very slow and small incremental increases in dosages as needed can circumvent the limiting factor of postural hypotension symptoms. This is the best way to successfully reach the more effective restorative effects with solid increases in LV systolic function.

Aside from hemodynamic benefits, the combination of BB + ACEI may be synergistic; and may depend upon their influence in helping to correct the immune system dysfunction that plays a pathogenic role in PPCM^[46-48]. Anticoagulation to avoid thromboembolic events is extremely important for those who have LVEF < 0.35 . In that lower cardiac function group it is important to monitor heart rhythm to detect and treat ventricular tachyarrhythmias.

Pentoxifylline, as an inhibitor of the proinflammatory cytokine, Tumor Necrosis Factor- α , appeared earlier in South Africa^[49] to be helpful to improve left ventricular function. However, in our trials in Haiti, pentoxifylline failed to show any evidence for improved survival or improved clinical or echocardiographic left ventricular function^[9,50].

Long-term follow-up is important as we continue to see late sudden death in some apparently recovered PPCM mothers; and do not know if this represents sudden cardiac death (SCD) and ventricular tachyarrhythmias as a consequence of PPCM-related scar tissue in the conduction system or from new onset disease, such as coronary artery disease^[51,52].

POST-PPCM PREGNANCIES

The majority of PPCM mothers who experience apparent full recovery (LVEF ≥ 0.50) will not experience a relapse of heart failure with a subsequent pregnancy; or, if they unexpectedly experience a relapse, the treatment, when initiated early, is very effective^[53,54]. In that case, the outcome is still good for mother and baby; and over 90% of those who begin the post-PPCM pregnancy with LVEF above 0.50 will recover to their pre-subsequent pregnancy cardiac function despite relapse^[54]. Risk of relapse of heart failure in a post-PPCM pregnancy increases incrementally in proportion to the systolic dysfunction associated with LVEF < 0.55 (Figure 2)^[54,55]. It is unclear what level of systolic dysfunction constitutes an absolute contraindication to a subsequent pregnancy; however, from extensive experience with post-PPCM pregnancies, it seems to me that the critical level is anything below LVEF 0.40^[54].

The published monitoring strategies^[53] are designed

to help assure early detection of relapse of heart failure, when effective treatment can bring about stabilization and offer excellent potential for another recovery of heart function^[51-56]. Although we may identify “full recovery” for PPCM as those with LVEF ≥ 0.50 , some of these women still go on to a relapse of heart failure in a post-PPCM pregnancy^[53-55] (Figure 2). That must mean that they really did not have a complete recovery or that they have a continuing reason for the development of pregnancy-associated heart failure; and we don’t yet know why. It is imperative to attempt to further identify the reasons for this, so that outcomes can still be satisfactory. Evidence supports the observation that even if these apparently completely recovered post-PPCM pregnancy mothers relapse, the treatment of their relapse of heart failure is very effective^[53,54].

The outcome is not nearly so good for post-PPCM pregnancy in those who have not reached the threshold of apparent recovery from the index episode of PPCM^[53,54]. We also do not know if prophylactic beta-blockade will prevent a relapse of heart failure with a post-PPCM pregnancy; or for that matter, if the BB might conceal early diagnosis of relapse, with delay of initiation of effective full treatment.

Even now, there are at least 3 observations that help us to distinguish “full recovery” from “apparent, but incomplete recovery”^[53,54]. First, an LVEF before subsequent pregnancy of 0.55 is a better indicator than an LVEF of 0.50 that the recovery is more likely to be successful without relapse of heart failure in another pregnancy (Figure 2). Secondly, a deterioration of LVEF with the gradual withdrawal of either BB or ACEI treatment is a good indicator that solid recovery has not yet occurred. Thirdly, inadequate contractile reserve on exercise stress echocardiography can be a predictor of likely relapse of heart failure in a post-PPCM pregnancy. With inadequate contractile reserve, it is better to defer subsequent pregnancy and strive for further improvement^[53,54]. It should be emphasized that a history of ventricular tachyarrhythmias warrants the continuation of BB treatment “for life”.

WORLD-WIDE PPCM

Pregnancy associated cardiomyopathy occurs globally, but with geographic variations for incidence, morbidity, mortality and unique characteristics. Cultural practices in Nigeria involving postpartum salt-loading and heated mud beds play an important role in the high incidence of heart failure, a variant of PPCM^[57]. High incidence of PPCM in Haiti seems to reflect the genetic influence of African heritage as well as micronutrient deficiencies, perhaps zinc, involved in immune system dysfunction^[26,58,59]. Overlap of PPCM and high incidence of HIV-disease appear to influence approach to PPCM in South Africa^[60]. Larger proportions of population with African heritage result in greater incidence and prevalence of PPCM^[40,61]. In China, common use of herbal remedies

may influence outcome for PPCM patients, but valid research is limited^[62,63].

WHAT INITIATES PPCM?

We do not yet know what is the actual “trigger” (there may be more than one) that initiates the process resulting in PPCM. This is perhaps the most difficult of all the quandaries about PPCM. We simply do not know. Some entertained the idea that fetal cells crossing into the maternal circulation may have targeted the mother’s heart (fetal microchimerism)^[28]. If anything, we now realize that these fetal cells may actually be helpful rather than harmful^[64]. Viral infection, as a trigger, has not been excluded; but neither has there been strong reinforcement of the likelihood. In personal files suggesting a possible link, I have identified 19 patients in whom the time framework of onset of new heart failure associated with pregnancy suggested a viral infection etiology (Table 3)^[65-70]. The largest of these studies^[66] showed similar incidence of the same viruses in endomyocardial biopsy tissue in both PPCM mothers and non-PPCM controls, making it unconvincing that virus played a role in those PPCM patients. It certainly seems likely that viral genomes in myocardial tissue may actually be “innocent bystanders” and not causal of disease, at least for some viruses. On the reverse side, it appears that for some cardiotropic viruses, once sensitization occurs, there may be an ongoing inflammatory process with or without viral genome persistence in the heart^[71].

In any case it seems likely that multiple triggers exist; often in the form of foreign antigens, serving in the role of “molecular mimicry”^[72,73], with epitope spreading, able to initiate an organ specific autoimmune disease^[28,72-74]. It is important to continue to put the pieces of the PPCM puzzle together and eventually the exact trigger or triggers will fit into the overall scheme of things. In the meantime, outcome results continue to improve, despite our lack of knowledge about actual trigger(s) for the process.

PPCM IN THOSE OF AFRICAN HERITAGE

We do not yet know why PPCM has been documented to be both more frequent and a more severe disease in those of African heritage^[13,17,31,75,76]. In the first North American prospective PPCM study, those with African heritage had a lower baseline LVEF and this poorer function persisted throughout the 12 mo study period^[17].

Harper *et al*^[24] identify the birth prevalence in North Carolina, United States, of PPCM for “black, non-Hispanics” as 1 case for every 1087 live births, four times the prevalence for “white, non-Hispanics” at 1 case for every 4266 live births. A California healthcare system reported the incidence of PPCM in blacks to be 1 case for every 1421 deliveries, 2.9 time higher compared with whites^[40]. Amos *et al*^[12], also identified a significant racial disparity in outcomes for PPCM in North Carolina, reporting that in their series of 55 PPCM patients, 51% of whom were

Table 3 Role of viral infection in the etiology of peripartum cardiomyopathy: Pathogenesis or mere presence?

ID	PPCM patient	Virus	Type of test	Comments
1	Author case file, Norway	Parvovirus B19	IgM/IgG + EMB + PCR	EMB = neg myocarditis
2	Case report, Italy ^[65]	Coxsackievirus B	IgM + blood PCR + blood	EMB = lymphocytic myocarditis
3	Case report, Germany ^[66]	Parvovirus B19	EMB + PCR	EMB = borderline myocarditis
4	Case report, Germany ^[66]	Parvovirus B19	EMB + PCR	EMB = borderline myocarditis
5	Case report, Germany ^[66]	E-B Virus	EMB + PCR	EMB = borderline myocarditis
6	Case report, Germany ^[66]	Human Herpesvirus 6	EMB + PCR	EMB = borderline myocarditis
7	Case report, Germany ^[66]	Human Herpesvirus 6	EMB + PCR	EMB = borderline myocarditis
8	Case report, Germany ^[66]	Cytomegalovirus	EMB + PCR	EMB = borderline myocarditis
9	Case report, Germany ^[66]	Parvovirus B19	EMB + PCR	EMB = inflammatory cardiomyopathy
10	Author case file, United States	Parvovirus B19	IgM/IgG + blood	Exposure to PVB19 child during pregnancy
11	Author case file, United States	Parvovirus B19 cytomegalovirus	IgG + blood	Hydrops fetus, stillborn
12	Case report, Japan ^[67]	Influenza A/B	Paired sera antibody rise	EMB = neg. Treatment with IV immunoglobulin
13	Case report, Japan ^[67]	Influenza B	Paired sera antibody rise	EMB neg. Treatment with IV immunoglobulin
14	Author case file, United States	Parvovirus B19	IgG + blood	Exposure to PVB19 child during pregnancy
15	Author case file, United States	Cytomegalovirus	IgM + blood	LVEF 15%, IgG + blood E-B virus
16	Case report, Taiwan ^[68]	PCR neg for all 4 tested	EMB/PCR neg, but myocarditis	2 mo pp, RV/LV failure, patient died VF
17	Author case file, United States	H1N1 Influenza	Nasal swab, no Rx given	LVEF 40% at Dx, day 1 postpartum
18	Case report, United States ^[69]	Parvovirus B19	EMB + PCR	HF 27 wk, g3p2 EMB neg myocarditis
19	Case report, Belgium ^[70]	E-B virus		Postpartum facial palsy full recovery 6 mo

EMB: Endomyocardial biopsy; PCR: Polymerase chain reaction; LV: Left ventricular; LVEF: LV ejection fraction; PPCM: Peripartum cardiomyopathy; PVB19: Parvovirus B19; RV: Right ventricular; VF: Ventricular fibrillation; HF: Heart failure.

“African American”, only 41% of African Americans recovered compared to 74% of “Whites”.

Goland *et al*^[75] recently reported a comparison of 52 African American PPCM patients with 104 white PPCM patients, finding that the rate of left ventricular recovery to LVEF ≥ 0.50 was significantly lower in African Americans (40% *vs* 61%; $P = 0.02$). This negative comparative outcome for those of African heritage has been also documented in Georgia^[75] and Louisiana^[13]. Gentry, in Georgia, United States, indicated that African-American women had a 15.7-fold higher relative risk of PPCM compared to non-African Americans (OR = 15.7, 95%CI: 3.5-70.6)^[76]. These outcomes in United States African American PPCM patients are more comparable to mortality and morbidity reports out of South Africa^[32] and Haiti^[26].

Significantly lower plasma levels of the proinflammatory cytokine, Transforming Growth Factor- β have been documented in both Haiti and South Africa^[32,33]. While it is possible that this is due to genetic factors, we cannot exclude a non-genetic environmental biopathological process. In either case this could result in worse outcomes. This factor has not yet been evaluated in African-American PPCM mothers compared to Caucasian or Hispanic mothers. While zinc deficiency resulting in immune system dysfunction is suggested as a possible nutritional factor in Haiti, this possibility awaits additional study; and certainly plays no role in nations where severe poverty is not an issue^[58,77].

It is important to promote further investigations of the previously mentioned differences in the postpartum

rate of restoration of NK cells in African heritage compared to Caucasian mothers^[31]. This may explain in part the lower diagnostic LVEF and the slower recovery rates found in these African-American mothers. It is possible that NK-T cells promote the expression of cardioprotective cytokines, such as Interleukin-10^[78]. An extra benefit of BB treatment may also be an increase in the percentage of NK T-cells, possibly partially correcting the disparity observed in African-American mothers^[30,79].

ROLE OF AUTOANTIBODIES IN PATHOGENESIS OF PPCM

We do not yet know how important a role cardiac autoantibodies play in PPCM. Are these autoantibodies, common in PPCM patients^[28,80], not only biomarkers of a cardiomyopathy, but also pathogenic in the process (Figure 3)? Some cardiac autoantibodies, such as the antibody targeting the β 1-adrenergic receptor, appear to be damaging to the heart^[81]. One of the most recent reports^[82] identifies autoantibodies against β 1-adrenergic receptors and M2-muscarinic receptors to correlate with worse cardiac systolic function. The finding of these serum autoantibodies also in 6/36 (16.7%) normal pregnant women, however, is troubling; and reinforces the need to follow such patients because they may not be actually “normal”^[83]. In our own studies, we found normal postpartum women to have none of the cardiac autoantibodies present in serum^[80].

Preliminary studies suggest that removal of these antibodies results in improved cardiac function^[84-86]. Per-

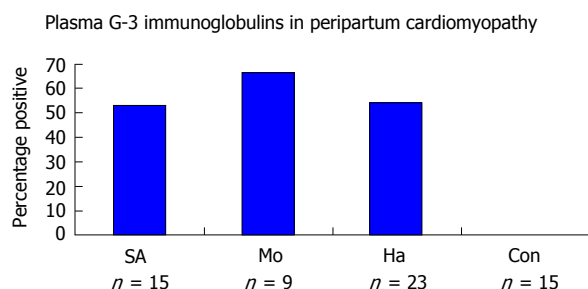


Figure 3 Autoantibodies in peripartum cardiomyopathy. Multiple types of cardiac antigen antibodies are common in PPCM. This Figure illustrates the presence of cardiac myosin heavy chain antibodies in PPCM patients from two African nations and Haiti. None were found in control normal postpartum patients from South Africa^[81]. SA: South Africa; Mo: Mozambique; Ha: Haiti; Con: Controls; PPCM: Peripartum cardiomyopathy.

haps the time has arrived for an interventional trial of immunoadsorption of these antibodies found in PPCM, particularly for those with low baseline LVEF, a group that is least likely to reach full recovery levels? This will not be easily accomplished because of the complicated procedure of apheresis and the precarious condition of the patients who could potentially be most helped by this process. An alternative that holds some promise of help is the use of peptides to neutralize putatively harmful cardiac autoantibodies, such as anti- β 1-adrenoreceptor antibodies, a much simpler process^[87,88].

ROLE OF PROLACTIN METABOLITES IN PATHOGENESIS OF PPCM

We do not yet know for sure that 14/16 kDa-prolactin metabolic products are cardiotoxic in humans; nor if inhibition of prolactin treatment produces better outcomes. As alluded to earlier, a strong foundation has been demonstrated for the cardiotoxic effects of “vasoinhibins”, the cleavage products of normal prolactin under situations of oxidative stress^[44,45]. However, studies to-date testing the effectiveness of prolactin inhibition treatment have given mixed results^[16]. Additional study with randomly-assigned PPCM patients to bromocriptine inhibition of prolactin cohort *vs* no bromocriptine inhibition treatment is underway and should help to clarify this potential treatment modality.

ROLE OF SOLUBLE FMS-LIKE TYROSIDE KINASE IN PATHOGENESIS OF PPCM

We do not yet know for sure that sFLT1 (also known as soluble vascular endothelial growth factor receptor-1) is cardiotoxic in humans; nor if inhibition of sFLT1 treatment will effectively promote the healing process. Soluble FLT1, a recently identified enzyme in the tyrosine kinase family, appears to be anti-angiogenic, cardiotoxic and particularly elevated in both PPCM and preeclampsia^[18,21]. If confirmed in larger series of PPCM patients, such as currently being addressed in the IPAC study, this may lead to

better treatments with promising anti-sFLT1 agents. With respect to preeclampsia, plasma sFLT1 has been found to be significantly elevated very early in some pregnancies, well before the clinical diagnosis of preeclampsia could be made^[89]. Early detection of plasma sFLT1 may also assist in confirming an earlier diagnosis of both PPCM and preeclampsia.

In particular, multiple groups of investigators are defining the clinical importance of finding the higher serum sFLT1/placental growth factor (PLGF) ratios^[19,89]. The highest ratios come about because of those with highest levels of serum sFLT1 (anti-angiogenic) and lowest levels of PLGF (pro-angiogenic) and it appears that this angiogenic imbalance can ultimately lead to heart failure^[88]. In this process, placental hypoperfusion and maternal endothelial dysfunction play important roles^[90]. This may turn out to be a very important development with respect to both diagnosis and management; but we are not yet certain. However, it is important to be alert to the possibility of peripartum heart failure from diastolic dysfunction, despite preserved systolic function with normal LVEF (would not meet current definition criteria for PPCM).

ROLE OF MICRONUTRIENTS IN PATHOGENESIS OF PPCM

Finally, we do not yet know if micronutrient and trace metal deficiencies play a role in the pathogenesis of PPCM in some unique situations. Earlier reports of endemic adolescent dilated cardiomyopathies due to selenium deficiency in China encouraged us to consider this possibility^[91,92]. In the high-incidence PPCM country of Haiti, we searched diligently for this possibility, but could not confirm it^[58]. However, further search led us to think that zinc deficiency could impact immune system functions and contribute to the process^[93-95]. Efforts to facilitate recovery with nutritional supplements in certain situations have provided some support; but remain unconfirmed and need further investigation^[96].

Please see Figure 4 with proposed multifactor hypotheses of the pathogenesis of PPCM. Case reports from the United States are included to illustrate some of the common serious complications that may accompany PPCM. These case reports come from the author's personal case file: PPCM Case Reports With Adverse Events: Note that all cases had diagnostic LVEF < 0.30.

Case 1 (United States): Onset with fetal distress and superior mesenteric artery thromboembolism

A 37 year-old gravida 4, para 2 patient presented in the 40th wk of pregnancy with swollen legs, mild dyspnea and fetal distress. She underwent emergency Cesarean section with rescued male infant. Post-operatively, she developed diffusely tender abdomen with absence of bowel sounds. Computed tomography scan of the abdomen suggested small bowel infarction. Chest X-ray revealed cardiomegaly, small right pleural effusion and increased pulmonary vascularity. An echocardiogram showed left ventricular

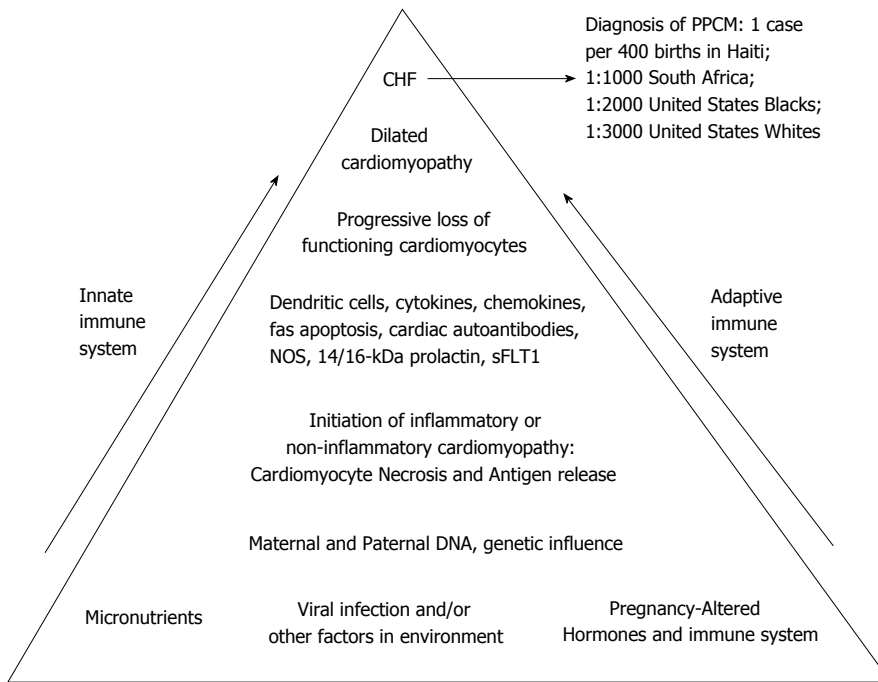


Figure 4 Schematic hypothesis for pathogenesis of peripartum cardiomyopathy. At the base of the pyramid are listed multiple potential contributing factors. Potential viruses include coxsackievirus B3, adenovirus, and parvovirus B19. Dendritic cells are activated by antigen(s) with initiation of a process leading to a cardiomyopathy that may be histologically either inflammatory or non-inflammatory. Cardiomyocyte damage results in the release of previously sequestered cardiac proteins with subsequent production of various autoantibodies, including but not limited to cardiac myosin heavy chain, cardiac Troponin-I, putative cardiac transaldolase), and cardiac beta 1-adrenergic receptor autoantibodies. Production of cytokines, chemokines, nitric oxide synthase (NOS) contribute to the negative inotropic effect. Fas-mediated apoptosis contributes to eventual cardiomyocyte loss. Ultimately, with the progressive loss of functioning cardiomyocytes, dilated cardiomyopathy and congestive heart failure (CHF) ensue, permitting a clinical diagnosis of PPCM. Both innate and adaptive immunity are involved, with participation of both cellular and humoral immune systems. Recently, other potential cardiotoxic substances have been identified, including 14/16 kDa-prolactin metabolites and kinase enzyme system, sFLT1^[21,26,28,32,33,37,38,44,63,66,70,77,80,93]. PPCM: Peripartum cardiomyopathy.

enlargement, end-diastolic diameter of 6 cm and LVEF of 0.17. Exploratory abdominal surgery confirmed necrosis of the small bowel, which was inoperable. She experienced circulatory collapse, cardiac arrest, and unsuccessful resuscitation.

Case 2 (United States): Onset with ventricular tachyarrhythmia, SCD

A 26-year-old gravida 1 patient in her 36th wk of pregnancy collapsed in her garage. She was found by family member who started cardiac cardiopulmonary resuscitation and called emergency services. Her cardiac rhythm normalized and she was taken to the hospital. Her echocardiogram showed mildly dilated left ventricle, end-diastolic diameter 5.1 cm, LVEF at diagnosis 0.17. There was an absence of fetal heart tones, with eventual vaginal delivery of stillborn; but the mother's heart function returned to normal over the next 6 mo.

Case 3 (United States): Onset with cerebrovascular thromboembolism

A 26 year-old gravida 2, para 1 patient in her 37th wk of pregnancy presented with paralysis of the right arm and leg (hemiplegia). Echocardiogram demonstrated thrombus in left ventricle, end-diastolic diameter left ventricle 5.4 cm, LVEF at diagnosis 0.15. Treatment included anticoagulation, hydralazine and metoprolol long-acting.

With stabilization of cardiac function, a Cesarean section was performed with birth of a healthy male infant. Heart function gradually normalized and one year later her only neurological deficit was mild weakness in right leg.

Case 4 (United States): Late diagnosis, chronic severe cardiomyopathy

A 20-year-old primipara developed preeclampsia in her last month of pregnancy. With stabilization of her blood pressure, a Cesarean section was carried out with the birth of healthy twins. She experienced postpartum edema, dyspnea, and abdominal pain. Abdominal ultrasound revealed cholelithiasis and laparoscopic cholecystectomy was performed. Post-operatively, she experienced more edema, dyspnea, and cough. She went to the Emergency Room twice, where blood tests showed abnormal liver function tests; Chest X-ray showed cardiomegaly, An echocardiogram demonstrated LVEF of 0.10. Her hemodynamic instability required a left ventricular assist device. Her LVEF persisted in the range of < 0.20 and she was placed on the transplant list.

Case 5 (United States): Subsequent pregnancy before recovery with eventual chronic dilated cardiomyopathy

A 31-year-old gravida 2, para 2 patient was diagnosed with PPCM two weeks postpartum with echocardiographic LVEF at diagnosis of 0.24. She received treat-

ment with lisinopril and carvedilol with improvement of her LVEF to 0.46. She phased out all medication and 3 years later became pregnant. She delivered a healthy female child; but subsequently experienced dyspnea on exertion and persistent pedal edema 3 d postpartum. An echocardiogram revealed reduction of echocardiographic LVEF to 0.34. She received treatment with lisinopril and carvedilol with gradual improvement of LVEF to 0.42, where it continued unchanged 3 years later.

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Cardioprotection and pharmacological therapies in acute myocardial infarction: Challenges in the current era

Alberto Dominguez-Rodriguez, Pedro Abreu-Gonzalez, Russel J Reiter

Alberto Dominguez-Rodriguez, Department of Cardiology, Complejo Hospitalario Universitario de Canarias, 38320 Tenerife, Spain

Alberto Dominguez-Rodriguez, Pedro Abreu-Gonzalez, Instituto Universitario de Tecnologías Biomédicas, 38320 Tenerife, Spain

Pedro Abreu-Gonzalez, Department of Physiology, University of La Laguna, 38320 Tenerife, Spain

Russel J Reiter, Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, United States

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Correspondence to: Alberto Dominguez-Rodriguez, MD, PhD, Department of Cardiology, Complejo Hospitalario Universitario de Canarias, Ofra s/n La Cuesta E-, 38320 Tenerife, Spain. adrvdg@hotmail.com

Telephone: + 34-922-679040 Fax: + 34-922-678460

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Abstract

In patients with an acute ST-segment elevation myocardial infarction, timely myocardial reperfusion using primary percutaneous coronary intervention is the most effective therapy for limiting myocardial infarct size, preserving left-ventricular systolic function and reducing the onset of heart failure. Within minutes after the restoration of blood flow, however, reperfusion itself results in additional damage, also known as myocardial ischemia-reperfusion injury. An improved understanding of the pathophysiological mechanisms underlying reperfusion injury has resulted in the identification of

several promising pharmacological (cyclosporin-A, exenatide, glucose-insulin-potassium, atrial natriuretic peptide, adenosine, abciximab, erythropoietin, metoprolol and melatonin) therapeutic strategies for reducing the severity of myocardial reperfusion injury. Many of these agents have shown promise in initial proof-of-principle clinical studies. In this article, we review the pathophysiology underlying myocardial reperfusion injury and highlight the potential pharmacological interventions which could be used in the future to prevent reperfusion injury and improve clinical outcomes in patients with coronary heart disease.

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Key words: ST-elevation myocardial infarction; Cardioprotection; Myocardial reperfusion injury; Infarct size; Adjunctive therapy

Core tip: As therapeutic interventions administered at the time myocardial reperfusion have been proven to reduce infarct size in both experimental and clinical models, the existence of a lethal reperfusion injury and its contribution to ischemic cardiac cell death can no longer be ignored. Patients presenting with an acute ST-segment elevation myocardial infarction will likely benefit from therapy aimed at the timely administration of drugs, most likely *via* primary percutaneous coronary intervention, for the reduction/prevention of lethal reperfusion injury. This approach will ensure that patients maximally benefit from the myocardial salvage that results from these therapies.

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INTRODUCTION

Acute myocardial infarction (AMI) is a major cause of mortality and morbidity worldwide. Each year, an estimated 785000 persons will have a new AMI in the United States alone and approximately every minute an American will succumb to one^[1]. In addition, AMI has major psychological and legal implications for patients and society and is an important outcome measure in research studies. The prevalence of AMI provides useful data regarding the burden of coronary artery disease and offers insight into health care planning, policy and resource allocation^[1].

The rapid time course of AMI and the temporal limitation on the maximal effectiveness of reperfusion constitute the pathobiological basis for the contemporary clinical strategies that emphasize early intervention within 1-2 h after the onset of symptoms^[2]. Currently, timely myocardial reperfusion using either thrombolytic therapy or primary percutaneous coronary intervention forms the cornerstone of treatment for acute ST-segment elevation myocardial infarction (STEMI) patients^[3]. However, mortality remains substantial in these patients, with in-hospital mortality ranging between 6% and 14%^[4].

Reperfusion profoundly alters the outcome of an evolving AMI. If instituted in a timely manner, a potential transmural AMI can be prevented and the extent of necrosis greatly reduced and limited to the subendocardium. However, some injured cardiomyocytes at the edge of the wavefront become irreversibly injured during the reperfusion phenomenon, producing a component of lethal reperfusion injury^[5]. After reperfusion, the salvaged myocardium exhibits impaired contractile function, a form of nonlethal reperfusion injury referred to as myocardial stunning. The earlier the reperfusion, the less total necrosis that occurs (including both the ischemia-induced and reperfusion-induced component), as well as the earlier the recovery of contractile function from the transient stunning. Conversely, reperfusion can be rendered less effective by the microvascular damage and obstruction that develop during the ischemic phase; this is known as the no-reflow phenomenon^[6,7].

In this minireview, we provide an overview of myocardial reperfusion injury and highlight potential pharmacological interventions for preventing it in reperfused STEMI patients.

PATHOPHYSIOLOGICAL MECHANISMS OF MYOCARDIAL REPERFUSION INJURY

There are various pathophysiological mechanisms involved in myocardial injury reperfusion. It has been suggested that mitochondrial permeability transition pore opening, overproduction of oxygen-derived free radicals and intracellular calcium overload might be candidates responsible for reperfusion injury. However, other factors of importance in the pathogenesis of reperfusion injury must be included, such as platelet and neutrophil-mediated injury, the renin-angiotensin system and the

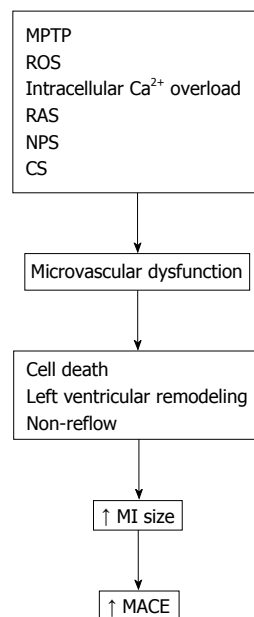


Figure 1 Scheme of mechanism of myocardial injury by a reperfusion process. MPTP: Mitochondrial permeability transition pore; ROS: Reactive oxygen species; RAS: Renin-Angiotensin system; NPS: Neutrophil-Platelet system; CS: Complement system; MI: Myocardial infarction; MACE: Major adverse cardiac events.

complement activation^[8,9] (Figure 1).

Mitochondrial permeability transition pore opening

Multiple lines of evidence have converged to show that the mitochondria have a central role in the pathogenesis of cell injury^[10,11]. In stressed cells, deleterious and salutary effects acting on mitochondria are mediated by death channels and salvage pathways, respectively^[12]. The mitochondrial death channels include the mitochondrial permeability transition pore and the mitochondrial apoptosis channel.

The mitochondrial permeability transition pore is a voltage-dependent channel that is regulated by calcium and oxidative stress^[13]. The opening in the first few minutes of reperfusion of the mitochondrial permeability transition pore, a non-selective channel of the inner mitochondrial membrane, in response to mitochondrial Ca^{2+} overload, oxidative stress, restoration of a physiological pH and ATP depletion, induces the cardiomyocyte death by uncoupling the biochemistry route of oxidative phosphorylation^[14], which leads to a reduction in ATP production.

Overproduction of oxygen-derived free radicals

Cell membranes are composed mostly of phospholipids and proteins. Alterations in membrane proteins by free radicals are among the important factors in the evolution of myocardial ischemia reperfusion damage. Large quantities of oxygen-derived free radicals lead to overwhelming of the cellular endogenous antioxidant defences. This causes, among other effects, the peroxidation of lipid membranes and loss of membrane integrity which results in necrosis and cell death^[15].

Re-introduction of abundant oxygen at the onset of reperfusion evokes a burst of additional toxic oxygen derivatives, including superoxide anion, hydroxyl radical and peroxynitrite, within the first few minutes of reflow. More-

Table 1 Pharmacological cardioprotective strategies for preventing myocardial injury in reperfused-ST-segment elevation myocardial infarction patients

Mitochondrial permeability transition pore opening	Overproduction of oxygen-derived free radicals	Intracellular calcium overload	Neutrophil-mediated injury	Platelet-mediated injury
Cyclosporin A	Adenosine	Glucose-insulin-potassium	Adenosine	Abciximab
Melatonin	Metoprolol	Atrial natriuretic peptide	Abciximab	Melatonin
	Erythropoietin Glucose-insulin-potassium Exenatide Melatonin		Erythropoietin Melatonin	

over, oxidative stress also reduces the bioavailability of nitric oxide (vasodilator compound) during reperfusion^[16].

Intracellular calcium overload

Changes in intracellular calcium homeostasis play an important role in the development of reperfusion injury. Intracellular calcium release at the time of myocardial reperfusion is mediated by damage to the sarcolemmal membrane and oxidative stress-induced dysfunction of the sarcoplasmic reticulum. These changes result in cardiomyocyte hypercontracture, mitochondrial calcium overload and the opening of the mitochondrial permeability transition pore^[17].

Complement system

The complement system is activated during reperfusion injury. This contributes to the formation of the anaphylatoxins C3a, C4a and C5a, as well as the terminal complement complex, the membrane attack complex, which is deposited in cell membranes. The complement factors induce direct cell injury by increasing cell permeability and release of histamine and platelet activating factor. In addition, complement factors, especially C5a, are potent stimulators of neutrophil adherence and superoxide production^[8].

Platelet and neutrophil-mediated injury

Neutrophils are important for the development of reperfusion injury by releasing oxygen free radicals, proteases and pro-inflammatory mediators that further amplify the infiltration of neutrophils into the jeopardized myocardium^[18]. Additionally, the hemorrhagic properties of neutrophils contribute to leukocyte entrapment in the capillaries, leading to microvascular plugging^[19].

Local platelet aggregation and deposition and also microembolization are partially responsible for reperfusion injury, especially in relation to microvascular dysfunction. Reperfusion injury induces platelet activation and this exacerbates the damage to the myocardium. Platelet products may exacerbate microcirculatory spasm, leading to further microvascular congestion, thrombosis and

sluggish coronary flow^[8,20].

Renin-angiotensin system-mediated reperfusion injury

The key product of the renin-angiotensin system, angiotensin II, increases intracellular calcium levels of cardiomyocytes and smooth muscle cells, leading to positive inotropism, impairment of diastolic function and coronary vasoconstriction. At pathophysiological levels, angiotensin II is cardiotoxic and induces myocyte necrosis^[8,21].

POTENTIAL PHARMACOLOGICAL THERAPIES FOR PREVENTING MYOCARDIAL REPERFUSION INJURY

Progress in understanding the basic pathobiology of ischemic heart disease has led to many years of research aimed at developing pharmacological approaches for limiting myocardial ischemic damage. Although myocardial ischemia-reperfusion injury is clearly mediated by several elements (Figure 1), agents aimed against these components of ischemic injury have not been consistently effective in different clinical trials^[2,22]. A number of reasons for the situation have been brought to light^[2,7,23,24].

A number of pharmacological interventions have been tried in the clinical setting to prevent myocardial reperfusion injury in reperfused-STEMI patients, although the results have been largely disappointing. Moreover, several pharmacological agents for preventing myocardial reperfusion injury in reperfused-STEMI patients are currently being tested in proof-of-principal clinical studies (Table 1)^[9,24].

Cyclosporin-A

Cyclosporine is known to inhibit the formation and opening of the mitochondrial permeability transition pore. In a proof-of-concept clinical trial involving 58 patients, cyclosporine administered as a 2.5 mg/kg intravenous bolus at the time of percutaneous coronary intervention was found to reduce the size of the myocardial infarct compared with placebo. Infarct size was reduced by 40%, as measured by creatine kinase release. Evaluation by magnetic resonance imaging also showed less myocardial damage^[25,26]. The ongoing CIRCUS study (NCT01502774) is investigating whether this therapeutic approach can reduce patient death, hospitalization for heart failure and a 15% increase in left ventricular end-diastolic volume.

Exenatide

Exenatide a new antidiabetic drug, has been shown to reduce myocardial infarct size by 23% of area at risk at 90 d, as assessed by magnetic resonance imaging, when given as an intravenous infusion started 15 min prior to primary percutaneous coronary intervention and continued for 6 h^[27,28].

Glucose-insulin-potassium

Of all the agents that have been tested reduce myocardial

infarct size or improve acute clinical outcome of STEMI, perhaps none is more controversial than glucose-insulin-potassium regimen. In the CREATE-ECLA trial, intravenous glucose-insulin-potassium infusion for 24 h was initiated after reperfusion of AMI. This trial had a negative outcome since it showed a difference in mortality at 30 d^[29]. The IMMEDIATE trial has been recently published. In this trial, the intravenous glucose-insulin-potassium infusion for 12 h was started by paramedics in the ambulance prior to reperfusion. The composite of cardiac arrest or in-hospital mortality was lower in 4.4% of glucose-insulin-potassium patients compared to 8.7% in the placebo patients ($P = 0.01$)^[30]. Thus, the use of glucose-insulin-potassium for AMI remains controversial and requires further studies.

Atrial natriuretic peptide

Kitakaze *et al.*^[31] demonstrated that an infusion of carperitide (an atrial natriuretic peptide analogue) during 72 h after reperfusion reduced myocardial infarct size and preserved left ventricular ejection fraction in reperfused-STEMI patients.

Adenosine

Two large multicenter studies, AMI Study of Adenosine (AMISTAD) 1 and AMISTAD 2, showed that a high-dose 3-h intravenous infusion of adenosine started near the time of reperfusion significantly reduced anterior wall myocardial infarct size, as determined by nuclear imaging^[32,33]. Other studies, however, were negative. A total of 112 patients with STEMI were randomized to 4 mg intracoronary adenosine or placebo. There was no benefit of adenosine on myocardial infarct size assessed by magnetic resonance imaging at 4 mo^[34]. Fokkema *et al.*^[35] also studied the effect of high-dose intracoronary adenosine boluses on myocardial infarct size and parameters of myocardial reperfusion. Four hundred and forty-eight patients with acute STEMI were randomized to placebo or 2 bolus injections of intracoronary adenosine. Adenosine did not improve the myocardial infarct size. Thus, the efficacy of the use of adenosine for AMI remains unproven and requires further studies.

Abciximab

In a recent study by Stone *et al.*^[36], 452 patients presenting within 4 h of STEMI with proximal or mid-left anterior descending coronary artery occlusion and undergoing percutaneous coronary intervention plus bivalirudin as an anticoagulant were randomized to bolus intracoronary abciximab, no abciximab, and to manual aspiration thrombectomy versus no thrombectomy in a 2 × 2 factorial design. The authors concluded that in patients with large STEMI undergoing percutaneous coronary intervention with bivalirudin, the addition of intracoronary abciximab bolus significantly reduced myocardial infarct size. Not all recent clinical trials with abciximab have been positive.

Thiele *et al.*^[37] compared intracoronary abciximab bo-

lus during primary percutaneous coronary intervention with an intravenous bolus in patients with STEMI. This large open-label, multicenter trial randomized > 2000 patients to intracoronary *vs* intravenous bolus abciximab followed by a 12 h intravenous infusion. The primary composite end point at 90 d (all-cause mortality, recurrent myocardial infarction or new congestive heart failure) was similar in the intracoronary group versus the intravenous group. Whereas the incidence of death and reinfarction did not differ between groups, fewer patients in the intracoronary group developed new congestive heart failure. The authors concluded that intracoronary abciximab bolus is safe and might be considered to reduce the rates of congestive heart failure. However, other secondary end points in this study, including enzymatic myocardial infarct size, were negative.

Erythropoietin

The large REVEAL study showed no reduction of infarct size^[38] and several other recent trials were negative for infarct size reduction^[39,40].

Metoprolol

The capacity of β -blockers to reduce infarct size was evaluated extensively in the pre-reperfusion era, with inconsistent results^[41]. In the context of reperfusion as the treatment of choice for STEMI, this has been poorly investigated. Experimental data suggest that the β -blocker metoprolol may reduce infarct size only when administered intravenously before reperfusion^[42,43].

Recently, the results have been demonstrated of the Effect of Metoprolol in Cardioprotection During an AMI trial, the first randomized, clinical trial prospectively evaluating the effect of early intravenous β -blockade on infarct size in conjunction with primary angioplasty. A total of 270 patients with anterior STEMI (Killip class II or less) revascularized within 6 h after symptom onset were randomized to receive intravenous metoprolol or not before reperfusion. All patients received oral metoprolol according to clinical guidelines (first dose, 12-24 h after infarction). Infarct size, evaluated by magnetic resonance imaging and creatine kinase release, was significantly reduced in the intravenous metoprolol group with no excess side effects. The left ventricular ejection fraction was higher in the intravenous metoprolol group^[44].

Melatonin

Melatonin, a circadian endocrine product of the pineal gland, is formed and released predominantly during night time. Melatonin has a diverse functional repertoire with actions in essentially all organs, including the heart and other portions of the cardiovascular system^[45-47]. Melatonin reduces the pathophysiological mechanisms that are involved in these benefits, in part due to the detoxification myocardial reperfusion injury, with respect to radical oxygen species and radical of oxygen and nitrogen-based reactants melatonin and its metabolites^[48,49]. Moreover, melatonin has indirect beneficial effects by increasing the

activity of principal antioxidant enzymes^[50]. Recent data also suggest that the mechanism of protection of melatonin appears to involve, at least in part, the inhibition of mitochondrial permeability transition pore opening *via* prevention of cardiolipin peroxidation^[51]. The lack of these cardioprotective effects due to insufficient high endogenous melatonin levels might be associated with several cardiovascular pathologies, including ischemic heart disease^[47,50,52].

Several studies show that humans with cardiovascular disease have noticeably lower circulating melatonin levels than age-matched subjects without significant cardiovascular deterioration^[53]. Recent investigations in patients with STEMI undergoing primary percutaneous coronary intervention confirmed a relationship between melatonin concentrations and ischemia-modified albumin, a biomarker of myocardial ischemia. These data suggest that melatonin can act as a potent antioxidant agent, reducing myocardial damage induced by ischemia/reperfusion^[54].

Because of the available scientific evidence, our group carried out a phase II clinical trial (ClinicalTrials.gov no. NCT00640094) using melatonin. We attempted to document whether intravenous and intracoronary melatonin administration reduces infarct size in STEMI patients treated by primary percutaneous coronary intervention by performing a multicenter, randomized, controlled clinical trial^[55]. The importance of these studies is emphasized by the fact that melatonin is quickly distributed throughout the organism when exogenously administered (oral, intravenous or subcutaneous). It crosses all morphophysiological barriers and enters cardiac cells with ease. The highest intracellular concentrations of melatonin are found at a mitochondrial level^[56]. This is especially important as the mitochondria is a major site of free radical generation and oxidative stress^[57].

Unless the findings in animal investigations are totally misleading, it is expected that melatonin will have similar protective effects benefitting the human heart. Melatonin is easily synthesized in a pharmacologically pure form and is inexpensive. Because of its marked versatility in protecting against oxidative stress and reducing inflammation in patients with myocardial ischemia, melatonin may have significant potential to improve public health.

CONCLUSION

A major determinant of post-infarction mortality and morbidity is the extent of myocardial necrosis after STEMI; therefore, strategies to limit infarct size are important. Several pharmacological interventions have been proposed as potential cardioprotective therapies but their use in clinical practice has been limited.

The list of cardioprotective agents that can be used as adjuvant therapy during to reperfusion is promising. Large multicenter clinical trials with enough statistical power will be necessary to establish the reported beneficial effects and to answer the question of whether they can improve clinical outcomes. To prevent translational

failure, particular attention must be paid to proper selection of patients (who will benefit the most), application (relevant concentration in the early phase of reperfusion) and hard end points.

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Shellfish allergy and relation to iodinated contrast media: United Kingdom survey

Mudassar Baig, Ahmad Farag, Jamal Sajid, Rahul Potluri, R Bruce Irwin, Hafiz Mohammed Idrees Khalid

Mudassar Baig, Lancashire Cardiac Centre, Blackpool Victoria Hospital, Blackpool, Lancashire FY3 8NR, United Kingdom
Ahmad Farag, Jamal Sajid, R Bruce Irwin, Hafiz Mohammed Idrees Khalid, Cardiology Department, Fairfield Hospital Bury, Pennine Acute Trust, Lancashire BL9 7TD, United Kingdom
Rahul Potluri, ACALM Study Unit in partnership with School of Medical Sciences, Aston University, Birmingham B4 7ET, United Kingdom

Author contributions: Baig M primary author performed the survey, literature search and wrote-up the article; Farag A designed the survey; Sajid J and Potluri R reviewed article and made further changes and suggestions; Irwin RB and Khalid HMI both were senior authors, made corrections and finalized the article.

Correspondence to: Dr. Mudassar Baig, Specialist Registrar (ST6) Cardiology, Lancashire Cardiac Centre, Blackpool Victoria Hospital, Blackpool, Lancashire FY3 8NR, United Kingdom. mudassarbaig@hotmail.com
Telephone: +44-798-0901673

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Abstract

AIM: To assess current practice of United Kingdom cardiologists with respect to patients with reported shellfish/iodine allergy, and in particular the use of iodinated contrast for elective coronary angiography. Moreover we have reviewed the current evidence-base and guidelines available in this area.

METHODS: A questionnaire survey was sent to 500 senior United Kingdom cardiologists (almost 50% cardiologists registered with British Cardiovascular Society) using email and first 100 responses used to analyze practise. We involved cardiologists performing coronary angiograms routinely both at secondary and tertiary centres. Three specific questions relating to allergy were asked: (1) History of shellfish/iodine allergy in pre-angiography assessment; (2) Treatments offered

for shellfish/iodine allergy individuals; and (3) Any specific treatment protocol for shellfish/iodine allergy cases. We aimed to establish routine practice in United Kingdom for patients undergoing elective coronary angiography. We also performed comprehensive PubMed search for the available evidence of relationship between shellfish/iodine allergy and contrast media.

RESULTS: A total of 100 responses were received, representing 20% of all United Kingdom cardiologists. Ninety-three replies were received from consultant cardiologists, 4 from non-consultant grades and 3 from cardiology specialist nurses. Amongst the respondents, 66% routinely asked about a previous history of shellfish/iodine allergy. Fifty-six percent would pre-treat these patients with steroids and anti-histamines. The other 44% do nothing, or do nonspecific testing based on their personal experience as following: (1) Skin test with 1 mL of subcutaneous contrast before intravenous contrast; (2) Test dose 2 mL contrast before coronary injection; (3) Close observation for shellfish allergy patients; and (4) Minimal evidence that the steroid and anti-histamine regime is effective but it makes us feel better.

CONCLUSION: There is no evidence that allergy to shellfish alters the risk of reaction to intravenous contrast more than any other allergy and asking about such allergies in pre-angiogram assessment will not provide any additional information except propagating the myth.

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Key words: Shellfish allergy; Contrast allergy; Iodinated contrast allergy; Low osmolarity contrast media; High osmolarity contrast media; Pre-angiography assessment

Core tip: This short survey explains how easily evidence base is missed out from real life practice. There has

never been any evidence to relate shellfish/iodine to contrast media, yet the myth been propagated for decades. Our survey gives a reminder and eye opener to change the practice to evidence base and thus helps in the patient care avoiding unnecessary medications.

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INTRODUCTION

There is a widely held view that a link exists between patient-reported shellfish allergy and increased risk of allergic reaction to iodinated contrast agents. Such agents are widely employed across many medical disciplines, including cardiology. Both invasive and non-invasive (in the case of computed tomography coronary angiography) diagnostic investigations require the use of such agents. Currently, guidance of percutaneous coronary angiography and many structural cardiac interventions mandates the use of iodinated contrast.

Historically the link between shellfish allergy and radio-contrast dates back to the early 1970s. Papers by Witten *et al*^[1] and Shehadi^[2] reported adverse reaction to radio-contrast in patients with history of seafood allergy. It is commonly believed that the individual with reported shellfish allergy is at higher risk of iodinated contrast allergy. It is often further assumed that this is due to the presence of iodine in both situations. Despite little evidence to support this relationship, many physicians still believe that shellfish/iodine allergy increases risk, and this may alter how such patients are treated. Various different methods of managing this perceived increased risk are currently employed, including prophylactic administration of corticosteroids or antihistamine preparations, and even avoidance of iodinated contrast altogether.

Our aims were to assess the current practice of United Kingdom cardiologists with respect to patients with reported shellfish/iodine allergy, and in particular the use of iodinated contrast for elective coronary angiography. Moreover we have reviewed the current evidence-base and guidelines available in this area.

MATERIALS AND METHODS

A questionnaire survey was sent by email to United Kingdom cardiologists. Both secondary and tertiary centres were targeted, as were multiple cardiologists within individual trusts. The aim was to establish routine practice amongst the surveyed cardiologists or specialist nurses for patients undergoing elective invasive coronary angiography. With this in mind, the three main questions posed were: (1) Do you ask about shellfish/iodine allergy history during pre-angiography assessment? (2) If patients

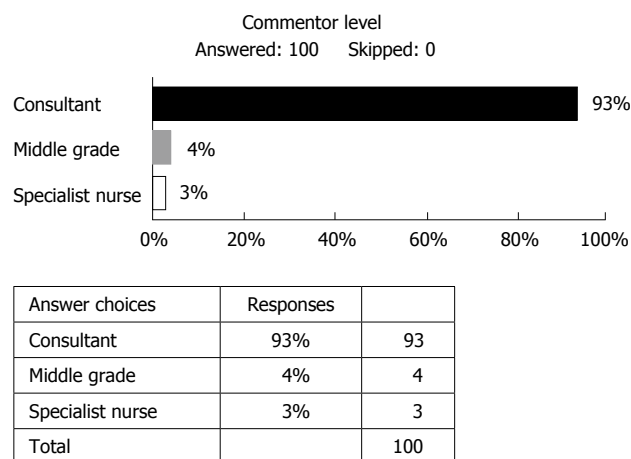


Figure 1 Level of commentor.

have history of shellfish/iodine allergy would you give pre-treatment? and (3) If pre-treatment is offered what is the protocol?

The physicians or specialist nurses completing the questionnaire were encouraged to elaborate and provide additional comments, as they felt necessary.

A comprehensive literature search was performed using PubMed. The following terms were used Shellfish Allergy, Iodinated contrast, and contrast allergy.

RESULTS

The questionnaire was sent to 500 cardiologists across the United Kingdom. A total of 100 responses were received, representing 20% of all United Kingdom cardiologists. Ninety-three replies (Figure 1) were received from consultant cardiologists, 4 from non-consultant grades and 3 from cardiology specialist nurses.

Amongst the respondents, 66% (Figure 2) routinely ask about a previous history of shellfish/iodine allergy while 56% would pre-treat these patients with steroids and anti-histamines (Figure 3). The other 44% do nothing, or do nonspecific testing based on their personal experience.

We found great deal of variation in practice with the following protocols followed: (1) Skin test with 1 ml of subcutaneous contrast before intravenous contrast; (2) Test dose 2 mL contrast before coronary injection; (3) Close observation for shellfish allergy patients; and (4) Minimal evidence that the steroid and anti-histamine regime is effective but it makes us feel better.

DISCUSSION

Shellfish allergy is one of the commonest food allergies in adults, and is a common cause of food-induced anaphylaxis^[3]. Seafood consumption has increased in popularity and frequency worldwide so as the adverse reactions^[3].

Shellfish can be classified into molluscs and arthropoda (crustaceans). Arthropods include crab, crayfish,

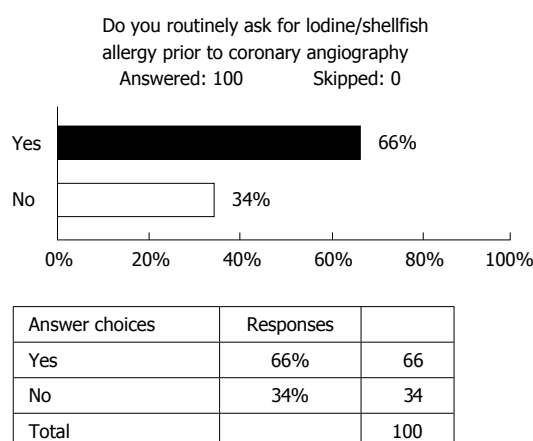


Figure 2 Number/percentage asking question about shellfish/iodine allergy prior to coronary angiography.

lobster, prawn and shrimp. Molluscs is subclassified into gastropod (abalone, conch, limpet, snail and whelk), bivalves (clam, cockle, mussel, oyster and scallop) and cephalopods (cuttlefish, octopus and squid). Four groups of allergens have been identified in shellfish: Tropomyosin, arginine kinase, myosin light chain and sarcoplasmic calcium-binding protein. Among the allergens identified, tropomyosin, a contractile protein, is considered a major allergen for prawn, and other crustaceans, such as shrimp, crayfish, lobster, crab and barnacles^[4].

The overall prevalence of shellfish allergy in the western world (United States, Canada and Europe) is approximately 0.6%, ranging between 0% to 10%^[5]. Of the shellfish, prawns are most frequently implicated (62% of shellfish allergy), followed by crab, lobster and then the molluscan species^[6]. Symptoms of shellfish allergy can range from mild urticaria to life threatening anaphylaxis. Most reactions are Immunoglobulin E (IgE)-mediated with rapid onset and may be gastrointestinal, cutaneous, or respiratory. Symptoms may be limited to transient oral itching or burning sensation within minutes of eating shellfish. Management of allergy is the same as for any other allergies *i.e.*, antihistamines, corticosteroids and adrenaline in severe or life-threatening reactions.

Shellfish allergy is mainly due to tropomyosins and iodine has in fact no role to play in allergic reactions. Moreover, iodine is an integral part of human body and essential for survival, therefore iodine itself cannot be considered an allergen. Radio-contrast media is composed of anions (iodide) and cations (sodium or meglumine). Iodine molecule is an effective X-ray absorber in the energy range therefore iodinated contrast media allow enhanced visibility of vascular structures and organs during radiographic procedures. There are two basic types of contrast media: ionic high osmolality contrast media (HOCM) and non-ionic low osmolality contrast media (LOCM), and both contains iodine molecule. HOCM (ionic) creates more charged particles and have more osmolality whereas LOCM (non-ionic) generates less dissociation and therefore have low osmolality. Examples of currently used ionic and non-ionic contrast media

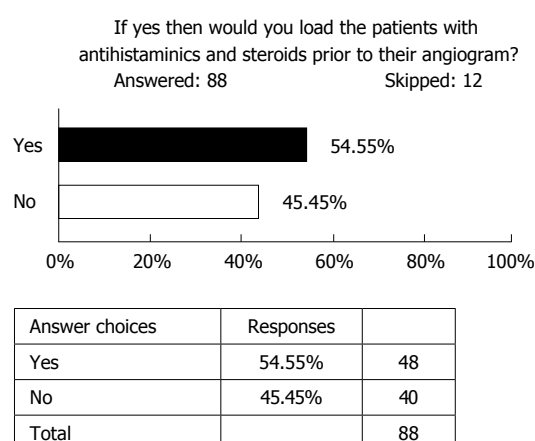


Figure 3 Patients treated with steroids and anti-histamines before coronary angiogram.

are: Perflutren-protein type-A microspheres injection (optison), iohexol injection (omnipaque), and non-ionic iodixanol injection (Visipaque).

Reactions to intravenous contrast are not truly allergic^[7] and not mediated *via* IgE. Instead there is direct stimulation of mast cells and basophils to release mediators leading to “anaphylactoid” reactions (pseudo-allergy). This may lead to urticaria, bronchospasm, hypotension, and even cardiac arrest. Previous allergic reactions to shellfish would create IgE sensitized to those allergens, but this sensitized IgE would play no role in allergic reactions to contrast media as they are not IgE mediated. Moreover, the cause of “anaphylactoid” reactions to contrast media is not the iodine in the contrast but is thought to be its hyper-osmolality compared to blood^[8].

Hyperosmolar contrast regardless of its composition is an irritant and will cause vasodilatation, increased vascular permeability, and direct cardiotoxicity and nephrotoxicity. Non-ionic contrast (LOCM) still uses iodine as a radiopacification agent, but fewer iodinated molecules are created with different side chains that reduce dissociation in solution. Fewer molecules in solution decrease the osmolality and therefore cause fewer side effects and reactions. These compounds are usually about one-half to one-third as osmotically active as the ionic forms^[9] and associated with fourfold or greater reduction in all adverse reaction and fivefold decrease in severe adverse reactions^[9]. The risk of reactions to intravenous contrast media ranges from 0.2%-17%, depending on the type of contrast used, the severity of reaction considered, and the prior history of any allergy^[9].

High risk patients include patients with previous intravenous contrast reactions, asthma, multiple true allergies, those taking beta-blockers or metformin, females, elderly and diseases that increase the risk of adverse reactions *e.g.*, pheochromocytoma, hyperthyroidism, thyroid cancer, renal failure^[10,11]. Atopy, in general, confers an increased risk of reaction to contrast administration, but the risk of contrast reaction is low, even in patients with a history of “iodine allergy”, seafood allergy, or prior contrast reaction. Allergies to shellfish, in particular, do not increase

the risk of reaction to intravenous contrast any more than of other allergies. A history of prior reaction to contrast increases the risk of mild reactions to as high as 7%-17%, but has not been shown to increase the rate of severe reactions^[9].

Mild reactions including warmth, nausea and vomiting occur only for short duration and do not require any treatment. Moderate reactions (*e.g.*, vomiting, sweating and swellings) occur in 1% of patients and frequently require treatment. Severe reactions occur in 0.02%-0.5% and deaths in 0.0006%-0.006%; neither has been related to "iodine allergy", seafood allergy, or prior contrast reaction^[9]. The most severe reactions, including death, have been reported to occur at similar rates with both types of contrast Media^[12].

Pre testing for contrast allergy is challenging and has been proposed in patients with a history of an anaphylactic reactions^[13]. Skin testing and Radioallergosorbent test have not been helpful in the diagnosis of contrast allergy as only a fraction of patients with severe reactions have a positive skin test^[14]. Small test doses are also not useful not only because severe reactions can occur even with small doses but also because of severe reactions to large doses of contrast media observed in patients who have tolerated small doses well. Therefore, no valid single test available to diagnose contrast allergy except only when symptoms occur after the contrast injection. However one can identify patients who are at high risk of contrast allergy^[15] and be prepared for adverse reactions.

Despite the increased use of non-ionic LOCM, and a decrease in the incidence of mild to moderate, and possibly severe reactions, pre-medications are still widely used in clinical practice. On the basis of observational data, Greenberger *et al*^[16] concluded in 1991 that patients with a previous reaction to high osmolality iodinated contrast media should receive oral prednisone and diphenhydramine with or without adrenaline. Since then, professional organisations have recommended a variety of regimens and combinations of methyl-prednisolone with or without an antihistamine^[17], oral prednisolone or methyl-prednisolone^[18], or intravenous hydrocortisone and intramuscular diphenhydramine^[19].

Steroid pre-medications reduce the incidence of respiratory symptoms due to contrast media from 1.4% to 0.4%, and the incidence of combined respiratory and hemodynamic symptoms from 0.9% to 0.2%^[20]. Thus, to prevent one episode of a potentially life threatening, iodinated contrast medium related reaction, about 100 to 150 patients need to receive steroids prophylactically^[20]. Disastrous anaphylactic complications after administration of iodinated contrast media seem to be rare. In the analysed trials, more than 10000 patients received an iodinated contrast medium but no reports of death, cardiopulmonary resuscitation, irreversible neurological deficit, or prolonged hospital stay was reported^[20].

Although it has been noted that steroid pre-medication decreases the total number of adverse events, it does not reduce the number of severe events. No significant effect is seen when steroids are given within 3 h before

administration of intravenous contrast media^[17]. Even with longer protocols, steroid premedication has not shown a statistically significant improvement in severe adverse reaction rates^[9].

For antihistamines, limited evidence shows that they may prevent some reactions. One may conclude that valid data supporting the efficacy of drug combinations or the use of premedication in patients with a history of allergic reactions are completely lacking^[20]. Severe allergic reactions due to contrast media seem to be rare; this may explain why no reports of disastrous reactions exist.

The treatment of an acute reaction to contrast media is no different from any other anaphylactic reaction. Treatment may include injectable epinephrine and antihistamines, as well as the use of intravenous fluids for low blood pressure and shock^[20].

In a conclusions, There is widespread variation in the management of patients who report previous shellfish allergy by United Kingdom cardiologists.

There is no evidence that allergy to shellfish alters the risk of reaction to intravenous contrast more than any other allergy, and this is due to: (1) Shellfish allergy is not related to iodine; instead the vast majority are due to tropomyosin; (2) Shellfish allergy is IgE mediated, whilst intravenous contrast allergy is due to direct stimulation of mast cells and basophils. Hence previous exposure to shellfish allergens and subsequently sensitized IgE, would play no role; and (3) Contrast pseudo allergic reactions are due to hyper-osmolality of contrast (free iodine molecule) rather than the bound iodine molecule.

It may be concluded therefore that there is no additional information gained by inquiring about previous shellfish/iodine allergy during pre-angiogram assessment. There is no specific relevance to this particular allergy, and such questioning potentially propagates the myth. If patients ask question about shellfish/iodine allergy they should be reassured and explained that there is no relation to contrast allergy.

There is no compelling evidence that anti-histamines have a role in prevention of allergic events, although corticosteroid pre-medication has shown benefit in reducing minor reactions, but no significant benefit in decreasing severe and fatal reactions.

It would be appropriate to use low osmolality, non-ionic contrast for patients with atopy, patients with previous reaction to intravenous contrast, and patients with systemic disease that increase their risk for contrast reaction. Almost all the life threatening reactions to intravenous contrast occur immediately or within 20 min of contrast injection so all patients with previous allergic reactions should be monitored and treat severe reactions the same way you would treat any severe anaphylactic reaction.

COMMENTS

Background

Radio-contrast is commonly used in both invasive and non-invasive diagnostic investigations but relation of the contrast media to shellfish and iodine allergy

is poorly understood.

Research frontiers

The authors conducted a questionnaire-based survey in United Kingdom to find out practice in relation to Shellfish/iodine allergy. They also looked at the current literature available and evidence base to establish the relationship between contrast media and shellfish/iodine allergy, if there was any.

Innovations and breakthroughs

The authors' survey found the more than 50% of the Cardiologists ask about shellfish/iodine allergy and pre-treat patients undergoing coronary angiography assuming that there exists a relation between the two. Looking at the evidence there is no such relation and by asking such questions in pre-angiography sessions they are propagating the myth.

Applications

The authors' research suggests no pre-treatment required for patient with history of shellfish/iodine allergy undergoing coronary angiography. This also prevents un-necessary medication use and stay in the hospital.

Terminology

LOCM: Low osmolality contrast media, HOCM: High osmolality contrast media, IgE: Immunoglobulin E.

Peer review

The present study showed at first the current practice of United Kingdom cardiologists with respect to patients with reported shellfish/iodine allergy, and in the use of contrast agent for elective coronary angiography. Second, the differences between shellfish and contrast allergy were explained in details including those mechanisms. Finally, the author stated the meaning of the pre-medication using antihistamines and/or steroids for the prevention of the contrast induced allergy. The suggestions in this manuscript seems to be very interesting, instructive and valuable, and the information in which may be of great use for many physicians in the real clinical setting.

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Steal syndrome secondary to coronary artery fistulae associated with giant aneurysm

Anastasia Vlachadis Castles, Tamara Mogilevski, Muhammad Asrar ul Haq

Anastasia Vlachadis Castles, Tamara Mogilevski, Muhammad Asrar ul Haq, Department of Cardiology, The Northern Hospital, Epping, VIC 3076, Australia

Muhammad Asrar ul Haq, Department of Medicine, University of Melbourne, Parkville, VIC 3010, Australia

Author contributions: Each of the listed authors contributed to drafting and revision of this manuscript.

Correspondence to: Dr. Muhammad Asrar ul Haq, Department of Cardiology, The Northern Hospital, 185 Cooper Street, Epping, VIC 3076, Australia. muhammad.asrar@unimelb.edu.au
Telephone: +61-3840-58000 Fax: +61-3840-58524

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Abstract

Giant coronary artery aneurysms and coronary artery fistulae are uncommon pathologies. We present the case of an elderly woman who was referred to cardiology for investigation of possible ischaemic heart disease prior to orthopaedic surgery. The patient had developed chest pain in the setting of a septic total knee replacement associated with changes on electrocardiography. Coronary angiography revealed multiple coronary arteriovenous fistulae associated with giant coronary artery aneurysm causing steal syndrome in the setting of haemodynamic stress.

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Key words: Coronary angiography; Coronary disease; Myocardial ischaemia; Coronary aneurysm; Vascular fistula; Chest pain

Core tip: This case report presents the angiographic findings of a rare occurrence of multiple coronary arteriovenous fistulae associated with giant coronary artery aneurysm and steal syndrome in the setting of haemodynamic stress.

INTRODUCTION

This case report presents the angiographic findings of a rare occurrence of multiple coronary arteriovenous (AV) fistulae associated with giant coronary artery aneurysms and steal syndrome in the settings of haemodynamic stress.

CASE REPORT

A 76-year-old lady was referred to cardiology for investigation of ischaemic heart disease (IHD) prior to orthopaedic surgery.

The patient was undergoing staged revision of a septic total knee replacement. After the first revision surgery, she experienced ischaemic chest pain. She had a history of chronic rate-controlled atrial fibrillation but no history of IHD. Her cardiovascular risk factors include hypertension, type 2 diabetes mellitus, age and post-menopausal status. There was no associated troponin rise however the electrocardiography revealed anterior T-wave inversion (Figure 1). Transthoracic echocardiography demonstrated mild tricuspid regurgitation, mild pulmonary hypertension, normal left ventricular function, and mild to moderately dilated right ventricle.

A coronary angiogram was performed *via* transradial approach. The study revealed two fistulae arising from distal left main coronary artery and proximal left anterior descending artery supplying a large aneurysm (Figure 2). The aneurysm, measuring 2.4 cm × 1.6 cm, drained into the pulmonary artery (PA) through multiple AV fistulae. The remainder of the coronary vasculature did not reveal any significant pathology.

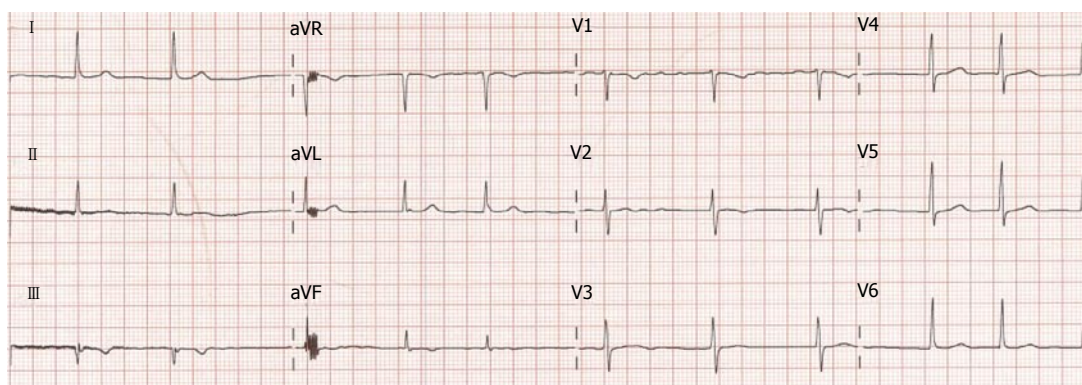


Figure 1 The patient's electrocardiography, demonstrating new anterior T wave inversion.



Figure 2 Coronary angiogram images demonstrating two fistulae arising from distal left main coronary artery and proximal left anterior descending artery supplying a large aneurysm. The aneurysm drains into the pulmonary artery through the arteriovenous fistulae.

In this case, the coronary artery aneurysm with associated fistulae was not deemed amenable to transcatheter closure/coiling given the size and multiple openings. Surgical repair was considered appropriate because of the risks of aneurysm rupture, endocarditis, thrombosis/embolism or heart failure^[1,2]. However the risk of significant periprocedural myocardial ischaemia secondary to upcoming orthopaedic procedure was small relative to the risk of undergoing cardiac surgery particularly in the context of underlying sepsis. The definitive management was therefore delayed until the patient's full recovery from sepsis and surgery.

DISCUSSION

Coronary artery aneurysms are fairly uncommon, being found in less than five percent of patients undergoing coronary angiography, most commonly in the right coro-

nary artery^[3,4]. Males are more commonly affected than females^[4]. The most common cause of coronary artery aneurysm in adults is atherosclerosis. As such, the same risk factors that predispose patients to atherosclerotic disease are also risk factors for coronary artery aneurysm formation^[3,4]. Other disease processes that damage the coronary arteries can also predispose to aneurysm formation; these include arteritis (infectious or inflammatory), syphilis, connective tissue diseases, Kawasaki's disease and metastatic malignancy. In addition, traumatic insult to the vessels, as in trauma, coronary angiography/intervention and aortic dissection, are implicated. Congenital malformations also increase the risk of developing coronary artery aneurysm^[3].

Coronary artery fistulae are rare, with an observed prevalence of less than one percent of patients on coronary angiography^[3-7]. Again, the right coronary artery is more commonly affected than the left coronary ves-

sels^[5,7]. The fistulae drain into the right cardiac chambers more commonly than the left, with the most common drainage sites being the right ventricle, right atrium or PA, and less frequently the coronary sinus, left atrium, left ventricle or superior vena cava^[5,7].

Coronary artery fistulae may be either congenital or acquired^[1,5-8]. Congenital fistulae may occur as an isolated anomaly or in conjunction with other congenital heart anomalies/malformations^[7]. Causes of acquired coronary fistulae include disease processes that damage the vessels, such as infection, inflammation and malignancy^[7,8]. In addition, trauma to the vessels, whether iatrogenic (as in cardiothoracic surgery and interventional procedures) or non-iatrogenic, may lead to fistula formation^[6-8].

Coronary artery aneurysm with associated fistula (CAAAF), being a combination of two uncommon pathologies, is extremely rare. As of 2005, only 50 cases had been reported^[1,4]. Little information is available about the aetiology of CAAAF but it has been observed that “most of the aneurysms were observed at the termination site of the fistulae”^[4].

Risks associated with CAAAF include aneurysm rupture, endocarditis, thrombosis/embolism, myocardial ischaemia and heart failure^[1,2]. Transcatheter, surgical or conservative management may be considered depending on the size, location and clinical context for the individual patient^[1,2].

COMMENTS

Case characteristics

An elderly lady with chest pain in the setting of sepsis.

Clinical diagnosis

Ischaemic heart disease was the most likely clinical diagnosis given the description of chest pain and the risk factor profile.

Differential diagnosis

Musculoskeletal or gastroesophageal reflux disease as exacerbation of chest pain with exertion could not be assessed given the patient's mobility was significantly limited by her orthopaedic condition.

Laboratory diagnosis

Serial troponin levels were negative.

Imaging diagnosis

Coronary angiography demonstrated multiple arteriovenous (AV) left main and left anterior descending coronary artery fistulae associated with giant aneurysm.

Treatment

The patient was medically managed for her hypertension, diabetes mellitus,

atrial fibrillation and ischaemic chest pain.

Related reports

Coronary angiogram images and electrocardiography are provided in the case report.

Term explanation

Coronary artery aneurysm refers to an abnormal dilatation of a coronary artery segment, relative to adjacent segments or other coronary arteries. Coronary artery (vascular) fistula refers to an abnormal connection between a coronary artery and another vessel or cardiac chamber.

Experiences and lessons

Coronary artery fistulae may only become symptomatic in the context of haemodynamic stress.

Peer review

The authors present a rare case report of multiple coronary AV fistulae with giant coronary artery aneurysms and steal syndrome. The manuscript is clearly written and well organized.

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**TOPIC HIGHLIGHT**

- 115 Coronary artery calcification in chronic kidney disease: An update
Stompór T
- 130 Myocardial ischemia is a key factor in the management of stable coronary artery disease
Iwasaki K
- 140 Clinical significance of glycated hemoglobin in the acute phase of ST elevation myocardial infarction
Lazzeri C, Valente S, Chiostrì M, D'Alfonso MG, Gensini GF
- 148 Duration of dual antiplatelet treatment in the era of next generation drug-eluting stents
Rha SW

REVIEW

- 154 Arrhythmogenic ventricular cardiomyopathy: A paradigm shift from right to biventricular disease
Saguner AM, Brunckhorst C, Duru F

MINIREVIEWS

- 175 High-sensitivity cardiac troponins in everyday clinical practice
Mair J

ORIGINAL ARTICLE

- 183 Molecular phenotypes of human parvovirus B19 in patients with myocarditis
Bock CT, Dückting A, Uitta F, Brunner E, Sy BT, Klingel K, Lang F, Gawaz M, Felix SB, Kandolf R
- 196 Coronary artery disease in congenital single coronary artery in adults: A Dutch case series
Said SAM, de Voogt WG, Bulut S, Han J, Polak P, Nijhuis RLG, op den Akker JW, Slootweg A

RETROSPECTIVE STUDY

- 205 Prognostic value of increased carbohydrate antigen in patients with heart failure
Méndez AB, Ordoñez-Llanos J, Ferrero A, Noguero M, Mir T, Mora J, Bayes-Genis A, Mirabet S, Cinca J, Roig E

CASE REPORT

- 213 Cardiac embolism after implantable cardiac defibrillator shock in non-anticoagulated atrial fibrillation: The role of left atrial appendage occlusion
Freixa X, Andrea R, Martín-Yuste V, Fernández-Rodríguez D, Brugaletta S, Masotti M, Sabaté M

Contents

World Journal of Cardiology
Volume 6 Number 4 April 26, 2014

APPENDIX I-V Instructions to authors

ABOUT COVER Editorial Board Member of *World Journal of Cardiology*, Niyazi Cebi, MD, Division for Vascular and Thoracic Surgery, Elbekliniken Stade, Stade 21682, Germany

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sion of Cardiology, Department of Medicine, University of California, Irvine, CA 92629, United States

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Xiu-Xia Song, Vice Director
World Journal of Cardiology
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: bpgoffice@wjgnet.com
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WJC 6th Anniversary Special Issues (2): Coronary artery disease

Coronary artery calcification in chronic kidney disease: An update

Tomasz Stompór

Tomasz Stompór, Department of Nephrology, Hypertension and Internal Medicine, University of Warmia and Mazury, 10-561 Olsztyn, Poland

Author contributions: Stompór T solely contributed to this paper. Correspondence to: Tomasz Stompór, MD, Department of Nephrology, Hypertension and Internal Medicine, University of Warmia and Mazury, 18 Zolnierska Str., 10-561 Olsztyn, Poland. stompin@mp.pl

Telephone: +48-89-5386219 Fax: +48-89-5337882

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Abstract

Arterial calcification is a well-recognized complication of advanced atherosclerosis. Chronic kidney disease (CKD) is characterized by significantly more pronounced, disseminated and fast-progressing calcification of the vascular system, including the coronary arteries. New computed tomography-based imaging techniques allow for the noninvasive assessment and monitoring of calcification in different vascular sites. Coronary artery calcification (CAC) develops early in the course of CKD and is tightly associated with mineral and bone disorders, which include but are not limited to secondary hyperparathyroidism. In this review, recent data on the pathogenesis of CAC development and progression are discussed, with a special emphasis on fibroblast growth factor 23 and its co-receptor, klotho. The prevalence, progression and prognostic significance of CAC are reviewed separately for patients with end-stage renal disease treated with dialysis, kidney transplant recipients and patients with earlier stages of CKD. In the last section, therapeutic considerations are discussed, with special attention paid to the importance of treatment that addresses mineral and bone disorders of CKD.

Key words: Chronic kidney disease; Dialysis; Kidney transplantation; Vascular calcification; Coronary artery calcification; Coronary artery calcification score; Agatston units

Core tip: Vascular calcification, a common feature of advanced atherosclerosis in the general population, is extremely advanced in patients with chronic kidney disease (CKD). CKD is associated with very fast progression of vascular (and in particular coronary) calcification. Pathogenetic aspects, clinical consequences and prognostic significance of coronary artery calcification in different CKD populations are discussed in this review. Therapeutic strategies used to limit the extent of vascular calcification and to improve the prognosis of patients with CKD are also discussed.

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INTRODUCTION

The importance of pathological calcification of soft tissue in chronic uremia has been recognized for a long time. The new era of research is associated with the introduction of new tools, allowing for noninvasive, quantitative assessment of mineral depositions in soft tissues, and electron-beam computed tomography (CT) and multi-slice CT (MSCT). A milestone study in the field was published in 1996 by Braun *et al*^[1] which documented an extremely high coronary artery calcium score (CACS) of 4290 ± 1509 Agatston units in patients on long-term hemodialysis (for comparison, a value of 400 Agatston units is associated with an extremely high risk of coronary artery disease in a general population). Many stud-

ies that followed this seminal paper reported advanced coronary and other cardiovascular calcification in patients with chronic kidney disease (CKD) in the pre-dialysis period, on hemodialysis, peritoneal dialysis and following kidney transplantation. Several studies also documented progression of arterial calcification in patients who remained on dialysis or progressed from earlier to more advanced stages of CKD. We were among the first who demonstrated such a progression in patients treated with peritoneal dialysis and attenuation of progression following kidney transplantation^[2-4]. Several experimental and clinical studies attempted to highlight mechanisms of development and progression of vascular calcification under the setting of chronic uremia. In this review, the pathophysiological background of coronary artery calcification (CAC) is discussed and the recent literature in the field of CAC in CKD reviewed.

CURRENT UNDERSTANDING OF PATHOPHYSIOLOGY OF CAC IN CKD

Calcium and phosphate

Mineral and bone disorders of CKD (CKD-MBD) develop early in the course of CKD. The hallmark of these disorders is hyperphosphatemia; levels of calcium and parathyroid hormone (PTH) are variable, *i.e.*, decreased, normal or elevated. Phosphate plays two important roles in the development of artery mineralization. It certainly serves as a substrate that is deposited within the tunica media or intimal layer of the vessel. It also acts as a mediator activating transcription of certain genes in vascular smooth muscle cells (VSMC) and pericytes which results in their transformation into osteoblast-like cells. The term “ossification” used sometimes with regards to pathological calcification is fully justified since this is not just a passive deposition of minerals within the vessel wall, but a precisely regulated process that mirrors bone formation. Macrophages resembling osteoclasts can also be found in an area of vascular mineralization; they become silenced upon challenge with phosphates, so the process of “bone formation” within the blood vessel is not counterbalanced with “bone resorption”^[5,6]. It should be emphasized that phosphate, considered a uremic toxin responsible for several adverse effects on cardiovascular system (CVS) in CKD, now has also been identified as such a toxin in the general population. Several population-based studies (such as the Framingham Offspring Study) showed that a high-normal serum phosphate level is also associated with a worse outcome and a higher risk of CV end-points^[7-9]. Low normal serum phosphorus in patients with normal renal function is associated with less calcification within coronary arteries^[10].

PTH

Changes in plasma PTH are linked to poor survival of patients with CKD, although the normal PTH level for a given level of glomerular filtration rate (GFR) is the matter of ongoing debate. Although recently published

Kidney Disease: Improving Global Outcomes (KDIGO) guidelines on CKD-MBD expanded the upper acceptable value in CKD stage 5 to as high as nine times above the reference value for normal subjects, recent studies indicate that mortality increases markedly when plasma PTH decreases below 150 or exceeds 300 pg/mL (according to most laboratories, the upper normal level for a healthy population oscillates around 70 pg/mL)^[11,12]. It seems that low plasma PTH is even more significantly associated with progression of vascular calcification than high PTH. Low bone turnover resulting from low PTH leads to decreased ability of bone to uptake calcium and phosphate delivered with diet since renal function is severely compromised and there is no “safety valve” by means of hypercalciuria and hyperphosphaturia; excess minerals activate pathological calcification and serve as substrates to this process^[13].

As in the case of phosphates, PTH is also considered cardiotoxic in uremia^[14,15]. High-normal plasma PTH is also considered a risk factor for increased CV morbidity in patients with normal renal function^[16,17].

Calcium sensing receptor

The discovery of calcium sensing receptor (Ca-SR) allowed for a more precise understanding of regulation of PTH synthesis and release in the course of calcium-phosphate metabolism disorders. Although its expression was originally thought to be limited to parathyroid cells, now it has become apparent that Ca-SR is present in several cell types. These include endothelial cells, cardiomyocytes and VSMC. Stimulation of Ca-SR on parathyroid gland cells strongly suppresses PTH synthesis and release. Ca-SR located in cardiovascular (CVS) structures seems to protect against their pathological calcification, decreased expression of this receptor observed in chronic uremia promotes osteoblastic transformation of VSMC and accelerates vessel wall calcification. Drugs designed to sensitize Ca-SR (*i.e.*, to enhance the receptor response even in lower serum calcium level, calcimimetics) were demonstrated to limit development and progression of vascular calcification in several experiments^[5,18-20]. This is in agreement with observations made in a general population suggesting that a high calcium diet is cardioprotective^[20]. Two distinct protective mechanisms of these drugs can be considered: better control of hyperparathyroidism and direct interaction with the vessel wall. Data from clinical studies using calcimimetics to control secondary (renal) hyperparathyroidism are equivocal, although these drugs tend to slow down the progression of coronary artery and heart valve calcification^[21].

Fibroblast growth factor 23 and klotho

The current era of investigation on vascular mineralization can be called the “era of Fibroblast growth factor (FGF)23 and klotho”. FGF23 was recently described as the hormone that acts as a strong phosphaturic agent in line with PTH. This protein is synthesized and released by osteocytes and represents the family of proteins re-

ferred to as phosphatonins. Both PTH and FGF23 are released upon stimulation by a high serum phosphate level. Although PTH and FGF23 act synergistically on the proximal tubular epithelial cells where they limit phosphate reabsorption (and thus enhance phosphaturia), their effects in other pathways is rather opposite. PTH enhances renal activation of active vitamin D (calcitriol) and thus increases intestinal absorption of calcium and phosphate; FGF23 decreases calcitriol synthesis and stimulates its degradation, in turn resulting in decreased GI absorption of calcium and phosphate^[22,23].

FGF23 starts to increase much earlier than PTH in the course of CKD. Its increase can already be noticed when the GFR decreases from 90 to 60 mL/min per 1.73 m²; thereafter, this increase is even steeper. Changes in serum calcitriol level follow FGF23. It starts to decrease when GFR falls below 60-70 mL/min per 1.73 m². PTH elevation is a rather late event; it occurs in the GFR range between 45 and 50 mL/min per 1.73 m². Increased serum phosphate can be noticed usually when GFR drops below 40 mL/min per 1.73 m²^[24]. This sequence of events indicates the efficacy of phosphaturic agents in elimination of phosphate *via* the kidney (they significantly increase single nephron phosphaturia which is sufficient to keep a normal serum phosphate level despite progressive loss of the total nephron number).

FGF23 has been identified as a very powerful predictor of poor prognosis, both all-cause and cardiovascular mortality. This predictive value applies to the whole population with CKD, including end-stage renal disease (ESRD), CKD stages 2-4 and kidney transplant recipients^[24-30]. FGF23 remains an independent predictive factor after correction for possible confounders, such as plasma phosphate, calcitriol or PTH. As in the case of high normal phosphate and PTH, borderline elevated or high normal FGF23 is also associated with a worse CV prognosis (this has been demonstrated, for example, in the Heart and Soul Study)^[31]. An association between CV outcome and plasma FGF23 can at least in part be explained by stimulation of vascular calcification; some data may indicate that this phosphatonin stimulates more tunica media calcification (Monckeberg calcification or arteriosclerosis that translates into increased arterial stiffness, left ventricular hypertrophy and heart failure) rather than intimal calcification (localized mostly within atherosclerotic lesions, atherosclerosis)^[32-35]. A predominance of Monckeberg-like lesions may in general explain why advanced CAC does not directly translate into coronary events (linked rather to calcification of lumen-narrowing atherosclerotic plaques). FGF23 was found to predict the severity of coronary artery disease in a large group of 1263 males and 813 females patients subjected to coronary angiography due to an acute coronary syndrome. FGF23 was an independent and strong predictor of stenosis score (that combined both severity of stenosis of an individual vessel and the number of vessels involved) and was also correlated with the extent of atherosclerosis and plaque calcification, as assessed with IVUS and vir-

tual histology. There were 368 patients with eGFR < 60 mL/min per 1.73 m². FGF23 appeared to predict the extent of stenosis and number of stenotic vessels (integrated together into stenosis score) in the whole study group and separately in patients with normal (> 60 mL/min per 1.73 m²) and reduced eGFR. FGF23 was inversely correlated with eGFR, but remained an independent predictor of coronary artery disease severity on angiography and the extent of atherosclerosis and plaque calcification on IVUS and virtual histology^[36].

Klotho is one of the most fascinating proteins discovered in relation to vascular calcification and FGF23 function. This protein is considered to have an important anti-aging potential and to protect against CVS disease^[37,38]. Since klotho is expressed mostly in renal tubular cells and parathyroid glands, this emphasizes the paramount importance of phosphate balance for cardiovascular health. Klotho facilitates normal phosphaturic function of FGF23 in the kidney and acts as its co-receptor. In experimental models of klotho, knock-out FGF23 loses its phosphaturic potential even if renal function is preserved. Renal content of klotho possibly decreases early in the course of CKD and triggers up-regulation of FGF23, even when other abnormalities of mineral balance (such as hyperphosphaturia) are not yet apparent^[39]. It is important to mention that several tissue receptors for FGF23 can be localized without klotho co-expression, possibly elevated FGF23 overstimulates these receptors leading to adverse CVS effects. Indeed, receptors for FGF23 can be found in cardiomyocytes and experimental studies demonstrate that FGF23 leads to left ventricular hypertrophy. This may suggest a direct cardiotoxic effect of FGF23^[32,33]. Klotho deficiency leads to increased expression of sodium-phosphate co-transporters Pit1 and Pit2 which facilitate phosphate transport into VSMC and stimulate their osteoblastic transformation. Runx2, a transcription factor that governs this transformation, is also upregulated in klotho deficiency^[40,41].

Vitamin D and vitamin K; matrix Gla protein

In many experiments, very high doses of vitamin D were shown to induce disseminated vascular calcification; these doses are never used in humans^[42]. Vitamin D receptor deficiency and a low vitamin D diet stimulate vascular calcification in mice^[43]. Experiments also demonstrated that vitamin D analogues [vitamin D receptor agonists (VDRA) modified in order to decrease their hypercalcemic effect] may protect against pathological calcification. Patients with CKD (and especially those with end-stage renal disease) suffer from profound vitamin D deficiency. Dietary regimes, lack of skin exposure to sun, failure to hydroxylate vitamin D in 1 α -position in failing kidneys, as well as the impact of high serum FGF23 contribute to such a deficiency^[44]. Low plasma level of 25-hydroxy-vitamin D is associated with poor survival in patients with ESRD and CKD, as well as with the risk of progression to ESRD^[45-47]. An association between low vitamin D status and adverse outcome in CKD may possibly be

explained in part by the risk of vascular calcification, inversely associated with plasma vitamin D (calcidiol)^[48]. Multiple clinical observational or registry studies demonstrated that supplementing 1 α -hydroxy-vitamin D is beneficial for the outcome of patients with end-stage renal disease; even better results can be achieved with novel analogues, such as paricalcitol. Unfortunately, these trials do not allow a conclusion of what the impact of vitamin D and other VDRA on vascular calcification in the clinical setting is.

Disseminated calcification of microcirculation that leads to necrotic lesions of skin and subcutaneous tissue, and ultimately to a fatal outcome has been well documented in ESRD (mostly on the level of case reports or case series) and is called calciphylaxis or calcifying uremic arteriolopathy (CUA). This phenomenon was demonstrated mostly in patients using warfarin and other drugs that antagonize vitamin K^[49,50]. Vitamin K is responsible for γ -carboxylation of several proteins, not only those of the clotting cascade. It contributes to post-translational modification of matrix Gla protein (MGP), a protein synthesized by VSMC which acts as a potent inhibitor of vascular calcification. This biochemical pathway was supposed to link development of CUA and the use of warfarin^[51,52]. Based on these observations, it has been hypothesized that vitamin K may have certain cardioprotective effects. The data from observational studies suggested a relationship between a higher intake of vitamin K (or biochemical measures suggesting high intake of this vitamin) and better CVS outcome, although a direct cardioprotective effect of vitamin K has not been proven to date^[53]. A high percentage of ESRD patients suffer from vitamin K deficiency; supplementing them with menaquinone 7 (vitamin K2) decreases the level of circulating uncarboxylated MGP. This observation may provide a rationale for the therapeutic use of vitamin K in order to prevent cardiovascular disease (possibly by limiting advancement of vascular calcification)^[54]. Low levels of carboxylated MGP were shown to predict a poor outcome in patients on maintenance dialysis^[55].

Inflammation

Chronic inflammation is a well-recognized factor that accelerates atherosclerosis and vascular calcification. Chronic inflammation is one of the hallmarks of uremia. It is triggered by the uremic status itself but also results from multiple co-morbid conditions activating inflammation (such as periodontal disease, activity of autoimmune systemic diseases, infection of vascular access for hemodialysis, presence of other foci of infection, *etc.*)^[56]. Several proinflammatory cytokines, such as interleukin 1, interleukin 6 or tumor necrosis factor alpha (TNF α), were shown to promote vascular calcification in experimental models of uremia and in uremic patients. C-reactive protein, the marker most commonly measured to assess inflammation, also correlated with the advancement of vascular and coronary calcification in patients with CKD^[3,4,57-60].

The anti-inflammatory potential of human serum seems to be essential in protecting patients against vascular calcification. One of the best recognized protective mechanisms is serum fetuin A. This is a “negative” (anti-inflammatory) acute phase protein synthesized by hepatocytes. It was hypothesized some years ago that fetuin A prevents precipitation of calcium and phosphate in serum. Uremic serum is supersaturated with calcium and phosphate, which suggests their ability to precipitate spontaneously in the absence of inhibitors. Fetuin A forms colloidal complexes with calcium apatite and other crystals (called calciprotein particles), thus preventing from their precipitation within soft tissues^[61]. Serum fetuin A was shown to predict prognosis in patients with advanced CKD; patient survival was inversely correlated with serum fetuin A^[62]. Recent years have brought new insight into the role of fetuin A in vascular calcification. Data concerning the association between serum fetuin A and soft tissue calcification are equivocal: some studies reported such an association, whereas others failed to demonstrate it^[63,64]. Hamano *et al*^[65] found, in an animal model of uremia and in humans with CKD, that centrifugation of serum at 16000 g can separate fetuin A into two fractions: pellets in sediment, containing fetuin A, fibronectin-1, albumin, fibrinogen, Ig κ light chains and Ig μ heavy chains; and apolipoprotein A-I and “free” fetuin fraction in supernatant. The pellets are also enriched with calcium. The authors found that the serum level of fetuin A before centrifugation is higher compared to supernatant fetuin A after centrifugation in patients with different stages of CKD (including ESRD and dialysis); such a difference was not observed in healthy controls. CACS did not correlate with fetuin A; however, it was correlated with the reduction ratio of fetuin A (*i.e.*, reduction in fetuin A level in supernatant after sedimentation, reflecting the amount of fetuin complexed with calcium and other proteins in the calciprotein particle). These results were confirmed and extended by Smith *et al*^[66], who also identified two fractions of fetuin in sera of patients with CKD, free and contributing to calciprotein particle formation. They found that high fetuin A in the calciprotein complex was positively associated with aortic pulse wave velocity, which reflects media calcification of arteries. In addition, they highlighted the importance of fetuin A molecule phosphorylation as a prerequisite to form calciprotein particles.

Epicardial fat as a new factor regulating CAC

Obesity and body mass index (BMI) were identified as important predictors of CAC both in the general population and in patients with CKD. Several cytokines such as TNF α that were implied in the development of CAC can be synthesized in adipose tissue; in addition, adipose tissue may be the source of more specific mediators (adipocytokines). The most important include leptin, adiponectin, visfatin and resistin. They were also shown to correlate with the degree and progression of CAC^[3,58,67]. Recently, a fascinating observation has been made, name-

ly, that similar to fat present in other body regions, epicardial fat is also characterized with certain metabolic and proinflammatory functions and the hormonal cross-talk between epicardial adipose tissue (EAT), myocardium and coronary artery exists^[68-73]. It is important to emphasize that adipose tissue in this location can be assessed quantitatively using similar techniques that are used to identify CAC (for example MSCT). Studies revealed an association between the amount of epicardial fat and the presence of CAC in post-menopausal women^[74]. Recently, the series of studies on such a link was published in CKD patients. Kerr *et al*^[75] searched for a correlation between CAC and epicardial fat volume in 94 stage 4-5 (pre-dialysis) CKD patients and found that CAC strongly and independently correlates with epicardial fat volume in this patient group. In addition, the amount of EAT was correlated with plasma interleukin 6, which confirms its inflammatory activity. A similar association was found in ESRD patients. Recent publications from the Turkish study group indicated that both CAC and EAT deposits were significantly more prevalent and more advanced in patients on renal replacement therapy compared to controls. These studies revealed an independent relationship between EAT and advancement of malnutrition, inflammation, atherosclerosis-calcification (MIAC) syndrome. MIAC integrates signs of malnutrition, enhanced “non-specific” inflammation of uremia, accelerated atherosclerosis and the presence of arterial calcification in one score. It cannot be concluded from the manuscript if there was a correlation between the amount of EAT and CACS^[76].

PREVALENCE AND PROGRESSION OF CAC IN DIFFERENT GROUPS OF CKD PATIENTS AND ITS ASSOCIATION WITH OUTCOME

In this part of the review, the recent, most important publications dealing with CAC and its clinical and laboratory associations in different groups of renal patients are discussed.

Dialysis patients

As mentioned previously, the phenomenon of an extremely advanced CAC was first identified and explored in patients treated with hemodialysis; these publications were followed by investigation in the field of peritoneal dialysis. In recent years, a series of publications were issued by the Italian independent study group. These authors aimed to analyze if randomization to different types of phosphate binders (sevelamer HCl *vs* aluminum or calcium-containing salts) have any impact on the progression of CAC. The study was performed in patients new to hemodialysis (which is important, since previously many were performed in prevalent patients, *i.e.*, with different dialysis vintage before inclusion). The 24

mo observation period was completed by 132 patients (23% diabetics); 70.4% had evidence of CAC at the study entry (although the initial CAC score was relatively low and equaled 286 ± 744 Agatston units). About 61% of patients experienced progression in CACS; it was independently and positively associated with the presence of diabetes, increasing serum LDL-cholesterol and C-reactive protein; randomization to sevelamer decreased the risk of progression by 34% ($P < 0.001$). This study also demonstrated that an increment in CACS correlates with progression of pulse wave velocity and worsening in cardiac repolarization, as measured with QT dispersion. As in most of the previous studies, it was also shown that baseline CACS is an important predictor of CACS progression; in contrast to several other studies, age did not predict the progression^[77,78].

High prevalence and fast progression of CAC were also identified in children and young adults with advanced CKD^[79,80]. This issue was analyzed recently by Srivaths *et al*^[81], who examined the relationship between CAC and FGF23, discussed above as one of the key predictors of cardiovascular outcome in renal patients. Sixteen patients aged 16 ± 3.3 years were involved in this study; they were on dialysis for quite a long period of time given their young age, *i.e.*, for 27.3 ± 19.3 mo. Compared to earlier reports on young patients, CACS was relatively low (median, 19; range 1-49 Agatston units) and present in only 5. FGF23 and serum phosphate were identified as being independently associated with CACS, although the statistical power in this small sized study must be considered very low. It should be emphasized that mean serum FGF23 level equaled 4024 pg/mL (in one of the recently published studies, the lowest quartile of FGF23 in patients with normal renal function was as low as < 40 pg/mL)^[36,81]. Pencak *et al*^[82], who recently analyzed correlations between CAC and a broad spectrum of calcification and bone turnover parameters (including FGF23, osteocalcin, osteoprotegerin, MGP, fetuin A, C-reactive protein, interleukin 6 and TNF α) in a large group of patients on hemodialysis, failed to reveal any association between CAC and any of the listed markers. Multiple logistic regression analysis allowed identification only of “classical” risk factors, namely age and time, on HD as independent predictors of CAC. FGF23 was not associated with the risk of CAC in the group of CKD patients (in stages 1-5) included in a recent Turkish study, although phosphatonic was related to valvular (aortic valve) calcification^[83].

The impact of CAC on survival was analyzed in hemodialysis patients included into the prospective Nutritional and Inflammatory Evaluation of Dialysis Patients study that comprised of 166 subjects on hemodialysis (51% diabetics) who were followed prospectively and all-cause mortality was analyzed according to baseline CACS. More than 80% of patients were Hispanic or black and the majority was dialyzed for more than 2 years. Patients were divided according to baseline CACS into four groups (0, 1-100, 101-400, 400+ Agatston units). There was a statistically significant trend towards increasing

age, percentage of diabetics and value of the Charlson Comorbidity score with increasing CACS category; no differences in serum calcium, phosphate, cytokine profile or BMI were observed between the groups. Fifty deaths occurred during follow-up: 30 in 400+ CACS group and only 2 in patients with CACS 0 at baseline. This translated into 88.9% event-free survival rate in patients without CACS compared to 58.3% in those with CACS 400+. Cox proportional regression analysis with adjustment for case-mix variables has shown that the hazard ratio of death in three CACS groups (1-100, 101-400 and 400+ Agatston units) equaled 2.9, 8.5 and 13.3 compared to the reference group (CACS = 0). This analysis also revealed that CACS measured for each coronary artery (individual CACS) was also predictive for all-cause mortality (with significance decreasing from the left main through left anterior and left circumflex to right coronary artery)^[84].

The predictive value of CAC for survival was also analyzed by the Italian group led by Prof. Gorgio Coen. 81 patients on maintenance hemodialysis for a very long time (82.5 ± 99.5 mo) at the time of baseline CAC assessment were included. In most of them (71 out of 81) CAC was found at baseline; the median value increased after one year from 481 to 528 Agatston units. Age and dialysis vintage were found to predict baseline CAC. A strong positive association was found between the baseline CAC and CAC increment over 12-18 mo observation period. In addition, calcium and PTH predicted the increment in CAC over this period of time, whereas fetuin A was shown to be protective. A total of 11 patients died during follow-up; mortality among those who progressed in terms of CACS increment equaled 72.7%. Agatston score was found to predict mortality during the follow-up^[85].

In many previously published studies, a fascinating link between CAC and bone turnover was postulated: in clinical circumstances with excess bone resorption, a certain amount of mineral content from the skeletal system may deposit within soft tissues, including the vessel wall. The inverse relationship between vascular calcification, vascular stiffness and bone mineral density was described in the general population^[86]. In CKD, characterized with bone and mineral disorders that are far more complicated than in osteoporosis, such a relationship was also documented^[87]. So called "adynamic" bone disease (low bone turnover) was postulated to be a form of bone mineral disorders that is frequently associated with advanced and progressing vascular calcification in CKD patients^[88]. Osteoprotegerin/receptor activator of NF- κ B ligand (OPG/RANKL) axis, crucial in regulation of bone resorption, was also postulated to be involved in pathological soft tissue calcification in uremia. The possible link between this axis and CAC was recently addressed in a group of 78 HD patients, 44 CKD stage 4 subjects and 42 healthy volunteers in a prospective manner. Serum OPG was significantly higher in HD patients compared to stage 4 CKD or healthy controls; an opposite trend could be seen for RANKL and resulted in a significantly

higher osteoprotegerin/RANKL ratio in HD patients compared to CKD stage 4 and healthy controls. Serum OPG and OPG/RANKL ratio were correlated with CAC at baseline and after one year; patients who progressed in CAC after one year (at least 10% and 50 Agatston units *vs* baseline) were characterized with a higher baseline and follow-up OPG and an increase in OPG during the one year observation period. Multivariate analysis confirmed an independent relationship between CAC progression and increase in serum OPG; high baseline CAC was also identified as another significant predictor of CAC progression. In the cited study, femoral bone mineral density was also measured but no correlation of BMD with baseline CAC or CAC progression was found^[89].

Pre-dialysis patients

The burden of CAC in CKD subjects not yet on dialysis is also significant, although generally less advanced compared to dialysis patients. The prognostic significance of CAC in pre-dialysis, however, was not known until recently. Russo *et al*^[90] analyzed the impact of baseline CAC and CAC progression on cardiac events in CKD patients not yet on dialysis (the study group comprised of the patients with CKD stages 2-5). They identified 181 patients with baseline CAC assessment who were followed prospectively and 54.7% of subjects were found to have CAC at baseline. The authors divided them into those with baseline CACS ≤ 100 and > 100 Agatston units and followed them until a cardiac event or end of the study, for a median period of 689 and 820 d, respectively (cardiac event was defined as cardiac death or myocardial infarction). Patients with higher baseline CACS were older, more frequently diabetic and had a longer duration of hypertension; interestingly, they did not differ in terms of GFR, mineral metabolism parameters, lipid profile or inflammatory markers. After adjustment for baseline differences, CACS > 100 Agatston units at the start of observation and accelerated progression of CAC (defined as annualized increment of CACS exceeding 75th percentile) were shown to predict cardiac events.

Another recent study addressed the issue of CAC progression in CKD patients not yet on dialysis. This study comprised of 103 CKD stage 3 and 4 patients with a baseline CAC assessment and who were then followed for 2 years. CAC was repeated after this period of time. Many other parameters, including a broad panel of biochemical markers and bone mineral density, were monitored. The study demonstrated that baseline CAC was higher in diabetic patients with CKD stage 3-4 compared to those without diabetes. Patients with diabetes were also more likely to progress in CAC compared to non-diabetics. The rate of progression was also faster among diabetics (although the increment in CAC was statistically significant within both groups). The prevalence of CAC greater than zero was also higher in diabetic CKD patients at baseline and follow-up (73% and 80%, respectively) compared to non-diabetics (46% and 60%). As in many previous reports, the most important predictors of

CAC progression were baseline CAC, BMI and serum phosphate level^[91].

Proteinuric patients

Proteinuria is considered a powerful predictor of cardiovascular events (CVEs) and mortality due to CVS disease. To the best of my knowledge, no study has been performed to analyze the prevalence or extent of CAC among patients with proteinuria in the course of primary kidney disease (primary glomerulopathy). However, a study was performed in diabetic patients with CKD and overt proteinuria (mean eGFR 52 ± 26 mL/min per 1.73 m^2 and median urine protein loss 2.7 g/g of creatinine, *i.e.*, close to nephrotic). No correlation was found between CAC and proteinuria, or eGFR; there was also no association between CAC and parameters of mineral metabolism, including calcium, phosphate, PTH or 25-hydroxy-vitamin D. Only age, male gender and ethnicity (being non-Latino white) were independently associated with advancement of CAC. In this study that involved 225 patients, 54 deaths occurred over the period of 39 ± 25 mo. CAC was an independent predictor of death in different statistical models and the hazard ratio of death equaled 1.49, 2.2 and 4.32 in patients with baseline CACS of 1-99, 100-399 and ≥ 400 Agatston units, respectively, compared to patients with CACS = 0^[92,93].

Renal transplant recipients

Several papers demonstrated that CAC is highly prevalent in transplant recipients and that successful kidney transplantation attenuates the rate of progression in CAC and mineralization within other vascular sites^[2,4,94,95]. Papers that were published recently expand our knowledge of CAC after kidney transplantation.

Shu *et al*^[96] analyzed the prevalence of CAC in a group of 99 renal transplant recipients from Taiwan. In 60% of patients CACS exceeded 10 Agatston units (mean and median values were not provided). CACS was independently associated with age and the presence of hypertension; female gender and high HDL-cholesterol were identified as protective factors in multivariate analysis.

Roe *et al*^[97] were among the first who analyzed the impact of CAC on CVEs and mortality in renal transplant recipients. These authors selected a broad spectrum of inflammatory markers in addition to other “classical” clinical and biochemical risk factors of CVEs. The study group consisted of 112 renal transplant recipients (31.5% diabetics, 61% received kidney from a deceased donor) with age a mean 48.8 ± 12.5 years. Dialysis vintage before transplantation was relatively short (3 ± 2.7 years). Mean calcification score equaled 367.7 ± 682.3 Agatston units (median 70.5 units, no CAC found in 38 patients). These results correspond with values expected in wait-listed dialysis patients (usually healthier compared to non-selected dialysis population). The patients ($n = 87$) had CAC assessment repeated after the median period of 1.7 years; in 25.9% CAC progression was noted and 95.1% of patients with CAC < 100 units survived, whereas survival

rate among those with CAC > 100 units was 82.3% ($P = 0.03$). The probability of remaining CVS event-free in respective CAC groups equaled 90.2% and 70.6%. Baseline CAC and CAC increments were shown to predict CVEs and mortality (depending on applied statistical approach, time spent on dialysis and if the presence of diabetes was predictive for CVS events or death).

Nguyen *et al*^[98] recently published the observation of 281 renal transplant recipients in whom initial CAC and aortic calcification were measured and the predictive value of arterial calcification in these two localizations on development of CVE was analyzed. The patients had a very long history of ESRD since the main dialysis vintage before transplantation was 2.4 ± 2.4 years and the time between transplantation and baseline CAC analysis equaled 8.3 ± 6.9 years. They were much younger than an “average” dialysis cohort (53 ± 13 years). Higher CACS and previously experienced CVE were identified as independent predictors of future CVEs during the mean observation period of 2.3 ± 0.5 years. These two factors combined significantly decreased the chance of remaining CVE-free during the follow-up. Interestingly, in this study, “classical” factors such as age, male gender, obesity, lipid profile disorders and smoking, did not predict the onset of CVE.

Seyahi *et al*^[99] analyzed the prevalence and progression of CAC in the group of renal transplant recipients a long time after transplantation (99.5 ± 54 mo) with well-preserved graft function (mean eGFR of 63.9 ± 18.1 mL/min per 1.73 m^2), who were earlier treated with dialysis for a mean period of two years. This Turkish population was much younger compared to an “average” Western dialysis or transplant cohort (38.7 ± 11.2 years) and, probably due to the young age, the prevalence and advancement of CAC was relatively low, despite a long history of renal replacement therapy (mean CACS 60 ± 174.8 Agatston units; median 0, range 0-1350; CAC present in 35.6% of patients). A very high percentage of patients (84%) received the kidney from a living donor. There were different methods of CAC progression defined in this study; depending on definition, progression in CAC was observed in 28%-38% of patients and prevalence of CAC-positive patients increased to 64.6% after 3 years. Baseline CAC and serum triglycerides were identified as independent predictors of CAC progression; in addition, bisphosphonate use was also independently associated with a 2.64-fold increased risk of CAC progression. The latter observation is very interesting and has been reported previously for other populations, for example, in a population-based Multi-Ethnic Study on Atherosclerosis. This study demonstrated that using bisphosphonates in post-menopausal osteoporotic women is associated with an increased risk of calcification in the aortic valve, aortic valve ring, mitral annulus, thoracic aorta and coronary arteries, especially in patients younger than 65 years^[100].

One of the most interesting studies in the field is the paper reporting prevalence and progression of CAC in transplant recipients who were on dialysis due to lupus

nephritis. Systemic lupus erythematosus (SLE) is one of the most important causes of “secondary” glomerular diseases, especially among young females, and certain types of lupus nephritis are associated with poor renal outcome and a need for renal replacement therapy. SLE is a systemic inflammatory disease with a very high risk of atherosclerosis and CVS disease^[101]. This includes a high prevalence of CAC in this patient group^[102]. Patients with SLE on dialysis are excellent candidates for kidney transplantation (unless no disease activity is observed at the time of transplantation) and the outcome after transplantation is comparable with non-SLE subjects. Hence the importance of study performed by Norby *et al*^[103] on CAC in renal transplant recipients should be acknowledged. These authors included 39 young renal transplant recipients with SLE (aged 34.1 ± 12.1 years, 74% female) in the study and identified a very high prevalence of CAC in MSCT (82%) and high mean and median CAC (894 ± 1679 and 135 Agatston units, respectively, with 36% of subjects with CAC exceeding 400 units). This important study identified the duration of SLE and BMI as independent predictors of CAC advancement; CAC was highly correlated with aortic pulse wave velocity (the measure of arterial stiffness and tunica media calcification). It should be emphasized that, in contrast to other papers in the field, the impact of dialysis on CAC in these patients was almost negligible: average time on dialysis was very short (13.2 ± 14.7 mo) and almost half of the recipients obtained a graft from a living donor^[103]. Given the fact that CAC was shown to predict cardiovascular outcome in transplant patients, it is, however, sad to say that these young people (predominantly women) can be considered as high-risk patients.

THERAPEUTIC PERSPECTIVE

There are only a few prospective randomized trials available in the literature with therapeutic interventions aimed at controlling cardiovascular disease and improving survival in patients with advanced CKD. Their general message is rather pessimistic since most of the trials failed to prove that therapeutic interventions really change outcome (exceptions include one small study with carvedilol in patients with ESRD and heart failure, and another large trial demonstrating benefits of combined treatment with simvastatin and ezetimibe *vs* placebo in advanced CKD)^[104,105]. Since there is an association between CKD-MBD, vascular calcification and mortality, mineral balance abnormalities became an obvious target for therapeutic interventions. Unfortunately, none of the interventions available in the field (including older and new phosphate binders, vitamin D and other VDRA, calcimimetics, low phosphate diet) was demonstrated to change patient prognosis and improve survival. This rather pessimistic notion was also upheld and emphasized by the most complex and comprehensive document in the field, namely, KDIGO clinical practice guidelines on CKD-MBD^[106]. Unfortunately, since publication of the KDIGO guidelines, no additional

data have been published to change this perspective. Probably the most disappointing news was the results of the EVOLVE trial; 3883 HD patients in this study were randomized to cinacalcet or placebo to test the hypothesis that treatment with cinacalcet would reduce the risks of death and nonfatal CVEs in this population. Unfortunately, no benefit was demonstrated from using the calcimimetic drug^[107]. Several other studies were performed to demonstrate the usefulness of certain drugs to reduce the advancement of vascular (and coronary) calcification or at least to slow down the progression over time.

Phosphate binders

The most obvious therapeutic intervention in CKD-MBD is using phosphate-binding agents to reduce absorption of calcium and phosphate from GI (and thus limit the availability of substrates and stimulating agents for vascular calcification). Since the drugs traditionally used for this purpose, namely calcium containing phosphate binders (usually calcium carbonate, calcium acetate and citrate), may be the source of additional and unwanted calcium supply (which may promote vascular calcification, limit possibilities of using vitamin D and lead to parathyroid gland oversuppression)^[108], most of the studies focused on the comparison between calcium-containing and calcium-free phosphate binders. The most important preparations in the field include lanthanum carbonate and synthetic compounds, sevelamer hydrochloride and sevelamer carbonate.

First, it is important to mention that in agreement with the KDIGO statement, other meta-analyses did not show survival benefit or attenuation in vascular calcification in patients using non-calcium containing phosphate binders *vs* those treated with calcium-based drugs^[109]. Thus, early enthusiastic reports on the positive impact of sevelamer on CAC progression or even mortality could not be confirmed; they were also criticized as being underpowered to detect any outcome differences and influenced by the pharmaceutical industry^[110-112]. In addition, other trials demonstrated similar efficacy of calcium acetate combined with a statin and sevelamer in control of CAC progression in patients on hemodialysis^[113]. The newer studies in the field point on the higher efficacy of sevelamer in limiting the progression of CAC compared to calcium-containing phosphate binders, although these publications are also statistically underpowered due to small study samples and relatively short observation periods. Shantouf *et al*^[114] found in a cross-sectional study that long-term sevelamer users on hemodialysis display lower values of CACS compared to those treated exclusively with calcium-containing phosphate binders. Barreto *et al*^[115] assigned treatment with sevelamer or calcium acetate to 101 HD patients and followed them for one year, with baseline and follow-up bone biopsy and CAC assessment. They failed to demonstrate any difference both in terms of changes in bone turnover and CACS progression over 12 mo between the two treatment groups. A randomized study completed recently in Japan

included 183 HD patients with a relatively long (118 ± 89 mo) history of dialysis. They were randomly assigned in a 1:1 ratio to sevelamer or calcium carbonate. CACS increased significantly in both treatment arms after one year (in both groups with P value of < 0.001 *vs* baseline), although the increase of CACS was significantly lower in patients using sevelamer after adjustment for baseline differences between groups^[116]. Similar results were also demonstrated for earlier stages of CKD. Russo *et al*^[117] randomized 100 patients with CKD 3-5 (in stage 5 patients not yet on dialysis) to low-phosphate diet only, sevelamer or calcium carbonate. A significant increase of CACS was noted after an average observation period of two years in patients randomized to diet only and calcium carbonate (in both groups with $P < 0.001$ *vs* baseline), whereas it remained stable in those using sevelamer hydrochloride. An annualized progression in CACS equaled 205 ± 82 Agatston units in controls, 178 ± 40 units in the calcium carbonate group and 36 ± 32 units in the sevelamer group^[117].

Sevelamer interacts with bile acid recirculation in the gut and may also influence lipid profile (with LDL-cholesterol lowering effect); some benefits of this polymer referred to this mode of action.

Lanthanum carbonate is a phosphate binder introduced to replace aluminum hydroxide in the treatment of hyperphosphatemia. In contrast to aluminum, GI absorption of lanthanum, a rare earth element, is considered negligible and thus it has been accepted as an effective phosphate binder without noticeable toxicity. In a recent study, it has been demonstrated that treatment with lanthanum carbonate is more effective compared to calcium carbonate in preventing the progression of CAC in patients on hemodialysis; in fact, regression by 6.4% was noticed in lanthanum-treated group *vs* 41.2% progression in those receiving calcium carbonate^[118].

Statins

The above mentioned study of Qunibi^[119] combined a statin with calcium acetate and demonstrated a similar efficacy in controlling CKD-MBD and CACS progression, as in the case of sevelamer. Lipid disorders are well-recognized triggers of atherosclerosis and they also contribute to arterial calcification^[119,120]. There were attempts to control CACS progression with statins, although the results are equivocal and today there is no scientific background to conclude that these drugs really stop CAC progression^[121-125]. Recently, Lemos *et al*^[126] randomized 117 patients with CKD stage 3 and 4 (eGFR 36 ± 16.5 mL/min) to treatment with rosuvastatin, sevelamer or control group and found no difference between the three groups in terms of CACS progression *vs* baseline after two years. Statins are widely used in the general population in both primary and secondary prevention. Data on the beneficial influence of statins on cardiovascular health in non-renal patients are extrapolated to CKD patients and most of them are treated with these drugs; there are also some preliminary data on the usefulness of the benefits

of statins in CKD^[105,127]. Hence, preventing CAC would probably not be the primary indication to commence these drugs in CKD patients since they are already widely used.

VDRA, vitamin K, cinacalcet

Although there is some pathological background to believe that low vitamin D status is associated with CAC progression, there are no clinical trials on the therapeutic role of vitamin D (native, calcidiol, calcitriol) in the prevention of CAC progression. The same holds true for paricalcitol, the leading vitamin D analogue which controls hyperparathyroidism with a less pronounced action on calcium and phosphate absorption from the gastrointestinal tract. The results of the most important recent trial testing the impact of cinacalcet on CAC progression are somewhat inconclusive. A total of 360 patients in this study (known as ADVANCE) were randomized to cinacalcet with vitamin D or to vitamin D alone. After 5 wk, CACS increased by 24% as measured in Agatston units and by 22% as measured using the volume method in cinacalcet users, whereas in the vitamin D group the respective increases equaled 31% and 30%. The difference between treatment arms was non-significant when values in Agatston units were compared but became significant ($P = 0.009$) when the volume scoring was applied. Cinacalcet significantly attenuated the progression of aortic valve calcification but had no influence on mitral valve and thoracic aorta^[21]. The results of this trial are difficult to interpret since VDRA were used in both treatment arms. ADVANCE was followed by publication of the EVOLVE trial, which demonstrated no impact of cinacalcet compared to placebo on mortality and major CVEs in the group of 3883 patients on maintenance dialysis^[107]. As mentioned above, although there are multiple publications on the role of vitamin K-dependent proteins in the development of vascular calcification, to date no interventional study has been performed to show the benefit of vitamin K treatment in slowing down the progression of CAC.

Bisphosphonates

The role of bisphosphonates in the treatment of CKD-MBD is unknown since classical osteoporosis is not included in the classification of this disease^[128]. In addition, a low value of GFR is a generally accepted contraindication for using these drugs. Small sample size trials performed in Japan some years ago suggested benefits associated with bisphosphonate use on CAC progression but it seems that this idea was abandoned since no further papers have emerged recently^[129,130]. As mentioned in this review, there is a link between bone metabolism and soft-tissue calcification. Osteoporosis as a main indication for bisphosphonates may per se promote vascular calcification since calcium and phosphate mobilized from bone may serve as a source of substrates. Bisphosphonates interact with vitamin K metabolism and thus may decrease γ -carboxylation of MGP, a well-recognized inhibitor of

pathological calcification. Specifically in patients with CKD (including moderate CKD after kidney transplantation), low-turnover bone disease develops which may be additionally worsened with bisphosphonates. These mechanisms may explain why the increased risk of calcification in the aortic valve, aortic valve ring, mitral annulus, thoracic aorta and coronary arteries was found in a substantial percentage of post-menopausal women using bisphosphonates to treat or prevent osteoporosis^[100]. On the other hand, bisphosphonates decrease expression of TNF α , down-regulate the inflammatory process and decrease the uptake of LDL-cholesterol by macrophages within atherosclerotic plaque; all these effects may potentially protect from calcification^[131].

CONCLUSION

Soft tissue and especially arterial calcification is a dangerous process which may affect patients from the general population but poses a special threat to subjects with chronic (advanced) kidney disease. Although many risk factors of the development and progression of arterial calcification were identified, they are not universally confirmed across studies; only age seems to determine CAC in all studies and baseline CAC usually determines its progression over time. The extremely complex nature of uremic toxicity, additionally complicated by treatment (dialysis or transplantation), makes the identification of a single or main modifiable risk factor extremely difficult. In an attempt to prevent the development and progression of CAC, several pathological pathways (mostly related to mineral and bone disorders) are targeted but due to multi-factorial etiology many others remain unaddressed. This results in a very high prevalence and fast progression of CAC in patients with CKD, with potential consequences in terms of increased cardiovascular morbidity and mortality.

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WJC 6th Anniversary Special Issues (2): Coronary artery disease

Myocardial ischemia is a key factor in the management of stable coronary artery disease

Kohichiro Iwasaki

Kohichiro Iwasaki, Department of Cardiology, Okayama Kyokuto Hospital, Okayama 703-8265, Japan

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Correspondence to: Kohichiro Iwasaki, MD, Department of Cardiology, Okayama Kyokuto Hospital, 567-1 Kurata, Naka-ku, Okayama 703-8265, Japan. kiwasaki@kyokuto.or.jp

Telephone: +81-86-2763231 Fax: +81-86-2741028

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Abstract

Previous studies demonstrated that coronary revascularization, especially percutaneous coronary intervention (PCI), does not significantly decrease the incidence of cardiac death or myocardial infarction in patients with stable coronary artery disease. Many studies using myocardial perfusion imaging (MPI) showed that, for patients with moderate to severe ischemia, revascularization is the preferred therapy for survival benefit, whereas for patients with no to mild ischemia, medical therapy is the main choice, and revascularization is associated with increased mortality. There is some evidence that revascularization in patients with no or mild ischemia is likely to result in worsened ischemia, which is associated with increased mortality. Studies using fractional flow reserve (FFR) demonstrate that ischemia-guided PCI is superior to angiography-guided PCI, and the presence of ischemia is the key to decision-making for PCI. Complementary use of noninvasive MPI and invasive FFR would be important to compensate for each method's limitations. Recent studies of appropriateness criteria showed that, although PCI in the acute setting and coronary bypass surgery are properly performed in most patients, PCI in the non-acute set-

ting is often inappropriate, and stress testing to identify myocardial ischemia is performed in less than half of patients. Also, some studies suggested that revascularization in an inappropriate setting is not associated with improved prognosis. Taken together, the presence and the extent of myocardial ischemia is a key factor in the management of patients with stable coronary artery disease, and coronary revascularization in the absence of myocardial ischemia is associated with worsened prognosis.

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Key words: Coronary artery bypass surgery; Coronary revascularization; Fractional flow reserve; Myocardial ischemia; Myocardial perfusion imaging; Percutaneous coronary intervention

Core tip: Studies of myocardial perfusion imaging demonstrate that, for patients with moderate to severe ischemia, revascularization is the preferred therapy for survival benefit. For patients with no to mild ischemia, medical therapy is the main choice, and revascularization is associated with increased mortality probably because of worsened ischemia. Studies using fractional flow reserve demonstrate that ischemia-guided percutaneous coronary intervention (PCI) is superior to angiography-guided PCI, and the presence of ischemia is the key factor in decision-making for PCI. Thus, myocardial ischemia is a key factor in the management of patients with stable coronary artery disease.

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INTRODUCTION

Coronary artery disease is a leading cause of mortality and morbidity in developing and developed countries^[1-5]. In approximately half of patients with newly diagnosed coronary artery disease, the first presentation is either acute myocardial infarction or sudden cardiac death^[6,7].

The development of percutaneous coronary intervention (PCI) has enhanced the management of patients with acute coronary syndrome, and the prognosis of these patients has been considerably improved^[8-15]. However, in patients with stable coronary artery disease, coronary revascularization decreases angina symptoms but does not significantly prevent cardiac death or myocardial infarction^[16-21]. Recent studies suggest that the presence and extent of myocardial ischemia determine the prognosis of patients with stable coronary artery disease. Coronary revascularization is associated with improved prognosis in patients with moderate or severe ischemia, but is associated with worsened prognosis in patients with no or mild ischemia^[22,23]. In this article, studies with myocardial perfusion imaging (MPI) and fractional flow reserve (FFR) on the effects of coronary revascularization on prognosis are reviewed.

CLINICAL OUTCOMES UTILIZING REVASCULARIZATION AND AGGRESSIVE DRUG EVALUATION TRIALS

Previous studies demonstrated that coronary revascularization does not significantly decrease the incidence of cardiac death and myocardial infarction in patients with stable coronary artery disease^[16-21]. In particular, the Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation (COURAGE) study had a tremendous impact on our management of patients with stable coronary artery disease^[24]. COURAGE trial is a randomized trial involving 2287 patients who had objective evidence of myocardial ischemia and significant coronary artery disease. The investigators assigned 1149 patients to undergo PCI with optimal medical therapy (PCI group) and 1138 to receive optimal medical therapy (OMT group) alone. The 4.6-year cumulative primary outcome (death from any cause and nonfatal myocardial infarction) rates were 19.0% in the PCI group and 18.5% in the OMT group (HR for the PCI group: 1.05; 95%CI: 0.87-1.27; $P = 0.62$). There were no significant differences between the PCI group and the OMT group in the composite of death, myocardial infarction, and stroke (20.0% *vs* 19.5%, HR = 1.05; 95%CI: 0.87-1.27; $P = 0.62$); hospitalization for acute coronary syndrome (12.4% *vs* 11.8%, HR = 1.07; 95%CI: 0.84-1.37; $P = 0.56$); or myocardial infarction (13.2% *vs* 12.3%, HR = 1.13; 95%CI: 0.89-1.43; $P = 0.33$). They concluded that as an initial management strategy in patients with stable coronary artery disease, PCI did not reduce the risk of death, myocardial infarction, or other major cardiovascular events when added to OMT.

However, the COURAGE Trial Nuclear Substudy tells another story^[25]. This study enrolled 314 patients who underwent MPI performed before treatment and 6 to 18 mo after randomization. At follow-up, the reduction in ischemic myocardium was greater with PCI than with OMT (-2.7% *vs* -0.5%; $P < 0.0001$). More PCI patients exhibited significant ischemia reduction (33% *vs* 19%; $P = 0.0004$), especially patients with moderate to severe pretreatment ischemia (78% *vs* 52%; $P = 0.007$). Patients with ischemia reduction had lower ischemia-unadjusted risk of death or myocardial infarction ($P = 0.037$; risk-adjusted $P = 0.26$), particularly if baseline ischemia was moderate to severe ($P = 0.001$; risk-adjusted $P = 0.08$). Death or myocardial infarction rates ranged from 0% to 39% for patients with no residual ischemia to $\geq 10\%$ residual ischemia on follow-up MPI ($P = 0.002$; risk-adjusted $P = 0.09$). Thus this study showed that adding PCI to OMT resulted in a greater reduction in ischemia compared with OMT alone, although the effect of PCI on death or myocardial infarction was borderline significant probably because of the small number of patients.

MPI

MPI is the most commonly used test to assess the presence and the extent of myocardial ischemia. Many studies demonstrated that the presence and extent of myocardial ischemia was closely related to adverse cardiac events^[26-36]. Hachamovitch *et al*^[36] identified 5183 patients who underwent MPI and were followed up for the occurrence of cardiac death or myocardial infarction. Over a mean follow-up of 642 ± 226 d, 119 cardiac deaths and 158 myocardial infarctions occurred, giving an annual cardiac death rate of 3.0% and annual myocardial infarction rate of 2.3%. In patients with no [summed stress score (SSS) 0-3], mild (SSS 4-8), moderate (SSS 9-13), and severe (SSS > 13) ischemia, the annual cardiac death rate was 0.3%, 0.8%, 2.3%, and 2.9%, respectively. Similarly, in patients with no, mild, moderate, and severe ischemia, the annual myocardial infarction rate was 0.5%, 2.7%, 2.9%, and 4.2%, respectively. Thus increased myocardial ischemia is associated with more frequent cardiac events.

Many studies also showed that coronary revascularization has a beneficial effect in patients with moderate to severe ischemia^[22,23,37]. Hachamovitch *et al*^[22] studied 10627 patients without known coronary artery disease who underwent MPI and were followed up for 1.9 ± 0.6 years. Within 60 d after MPI, 671 patients underwent revascularization therapy and 9956 patients underwent medical therapy (MT). On the basis of the Cox proportional hazards model predicting cardiac death, patients undergoing MT demonstrated a survival advantage over patients undergoing revascularization in the setting of no or mild ischemia (% total myocardial ischemia less than 10%), whereas patients undergoing revascularization had an increasing survival benefit over patients undergoing MT when moderate ischemia (% total myocardial ischemia 11%-20%) to severe ischemia (% total myocardial ischemia more than 20%) was present. In 2011, the same

authors expanded their sample to 12329 patients and studied the interaction between the extent of ischemia and myocardial scar after revascularization on patient survival^[23]. In the absence of prior coronary artery disease, increasing amounts of ischemia were associated with lower HRs with early revascularization. In the setting of little or no ischemia, early revascularization was associated with an approximately 50% greater risk than MT, whereas, with increasing ischemia, a progressive improvement in risk with early revascularization compared with MT was found. In the setting of extensive ischemia (> 20% myocardium), a 30% reduction in risk of all-cause death was present with the use of early revascularization compared with MT. Equipose between the two strategies was present with approximately 10%-15% of the myocardium ischemic. As for patients with < 10% fixed defect, the risk reduction was 12.5% with MT and for patients with prior revascularization but no prior myocardial infarction it was 7.5%. Thus, these studies demonstrate that for patients with moderate to severe ischemia, revascularization is the preferred therapy for survival benefit, whereas for patients with no to mild ischemia MT is the main choice and revascularization is associated with increased mortality.

WHY IS CORONARY REVASCULARIZATION IN PATIENTS WITH NO OR MILD ISCHEMIA ASSOCIATED WITH INCREASED MORTALITY?

There is some evidence that revascularization in patients with no or mild ischemia is not associated with improved ischemia, but rather associated with worsened ischemia. Safley *et al*^[38] identified 301 patients who underwent PCI for chronic total occlusion and in whom MPI was performed within 12 ± 3 mo before PCI and a follow-up study within 12 ± 3 mo after PCI. The change in % ischemia was +5.39% ($P = 0.006$), -1.70% ($P = 0.008$), -6.32% ($P < 0.001$), and -16.26% ($P < 0.001$) in patients with no/minimal (< 5% ischemic myocardium), mild (5%-9.9%), moderate (10%-16%), and severe (> 16%) ischemia, respectively. The percentage of patients with improved ischemic myocardium $\geq 5\%$ was 0%, 34.7%, 68.5%, and 86.7% in patients with no/minimal, mild, moderate, and severe ischemia, respectively ($P < 0.001$). The percentage of patients with worsened ischemic myocardium $\geq 5\%$ was 87.3%, 34.7%, 19.2%, and 9.2% in patients with no/minimal, mild, moderate, and severe ischemia, respectively ($P < 0.001$). Kaplan-Meier survival in patients with *vs* without improvement in ischemia showed a survival advantage in patients with improved ischemic myocardium $\geq 5\%$ (87% *vs* 78%, $P = 0.018$). Receiver operating characteristics curve (ROC) analysis identified a 12.5% ischemic burden as the optimal cut-point to predict improvement in ischemia following PCI (sensitivity 80%, specificity 80%). This 12.5% ischemic

burden is almost the same as that in the 2011 study by Hachamovitch *et al*^[23]. Also ROC analysis identified a 6.25% ischemic burden as the optimal cut-point to predict worsening in ischemia following PCI (sensitivity 75%, specificity 80%). Thus, this study demonstrated that revascularization had no survival benefit and harms patients with no to mild ischemia, although the study was limited to patients who underwent PCI for chronic total occlusion.

Myocardial infarction associated with PCI (periprocedural myocardial infarction) is classified as type 4a by the third universal definition of myocardial infarction^[39]. The prevalence of periprocedural myocardial infarction is 7.3% to 17.9% defined by CK-MB isoenzyme elevation > 3x upper limit of normal (ULN) and 15.0% to 44.2% defined by cardiac troponin > ULN^[40-55]. The results of several studies suggested that any elevation in CK-MB was associated with reduced long-term survival and that there was a direct correlation between the magnitude of myonecrosis and mortality. Other studies have shown that only large myocardial infarctions were predictive of a poor long-term outcome^[40-46]. Similarly, some studies showed that the serum concentration of cardiac troponin was an independent predictor of survival, others did not^[47-55]. However two recent meta-analyses concluded that an elevated cardiac troponin levels after PCI does provide prognostic information^[56,57]. Risk factors of periprocedural myocardial infarction are those which identify patients with increasing atherosclerotic disease burden, increased thrombotic risk, and with neurohormonal activation that predispose to either macrovascular complications (side branch occlusion or macroembolization) or microvascular obstruction (distal embolization of microparticles)^[58].

In the era of coronary angioplasty, many studies reported that numerous "false positive" reversible perfusion defects occurred early after angioplasty, possibly as a result of inadequate early vessel remodeling or sustained abnormalities of coronary vasomotor tone. However, a significant percentage of patients showed persistent abnormalities in the later period^[59,60]. In one study, 76% of patients without prior myocardial infarction showed improvement in perfusion abnormalities after angioplasty, but only 34% had completely reversible ischemia^[60]. In the other study of 15 patients 1 to 2 wk after angioplasty, 7 had a reversible perfusion defect, of whom only 4 subsequently normalized by 4 to 6 wk^[61]. These studies suggested that an improved or normalized perfusion abnormality does not necessarily occur after coronary angioplasty in every patient. Taken together, revascularization in patients with no or mild ischemia is likely to result in worsened ischemia, which is associated with increased mortality.

ISCHEMIA-GUIDED REVASCULARIZATION

There are some studies which showed that the ischemia-

guided (IG) strategy resulted in a better prognosis^[67-70]. Farzaneh-Far *et al*^[67] identified 1425 consecutive patients with coronary artery disease who underwent two serial MPI. They were followed for a median of 5.8 years after the second MPI. Patients were included in the PCI or coronary artery bypass graft (CABG) group on the basis of the first revascularization procedure occurring within 60 d of the first MPS scan. Thus patients were divided into a MT group, PCI group, and CABG group. The incidence of patients with worsening of the ischemic myocardium by $\geq 5\%$ was more frequent in the MT group (15.6%) compared with the PCI (6.2%) and CABG groups (6.7%) ($P < 0.001$). After adjustment for established predictors, $\geq 5\%$ ischemia worsening remained a significant independent predictor of death or myocardial infarction (HR = 1.634; $P = 0.0019$). Thus, this study showed that ischemia worsening was an independent predictor of death or myocardial infarction, and revascularization was associated with more frequent improvement in myocardial ischemia compared with MT.

Kim *et al*^[68] studied the importance of IG revascularization. From a registry of 5340 patients with multivessel coronary artery disease, comprising 2587 PCI and 2753 CABG. MPI was performed in 42.3% of patients and IG revascularization was performed in 17.3%. The MPI was defined as abnormal if the SSS was 3 or greater. The incidence of major adverse cardiac and cerebrovascular events (MACCE) was significantly lower in the IG group than in the non-IG group [16.2% *vs* 20.7%, adjusted HR (aHR) = 0.73; 95%CI: 0.60-0.88; $P = 0.001$], primarily driven by the lower repeat revascularization rate (9.9% *vs* 22.8%, aHR = 0.66; 95%CI: 0.49-0.90; $P = 0.009$). Subgroup analysis showed that IG reduced the risk of MACCE in PCI patients (17.4% *vs* 22.8%, aHR = 0.59; 95%CI: 0.43-0.81; $P = 0.001$) but not in CABG patients (16.0% *vs* 18.5%, aHR = 0.87; 95%CI: 0.67-1.14; $P = 0.31$). Thus IG revascularization with MPI, particularly in PCI-treated patients, seems to decrease the risk of repeat revascularization and MACCE in patients with multivessel disease. Taken together, these studies suggest that the IG strategy is associated with improved prognosis.

FFR

FFR (the ratio of maximal blood flow in a stenotic artery to normal maximal flow), is now a gold standard for invasive assessment of coronary artery stenosis^[71-80]. In Fractional Flow Reserve *vs* Angiography in Multivessel Evaluation (FAME) study, investigators randomly assigned 1005 patients with multivessel coronary artery disease to PCI with implantation of drug-eluting stents guided by angiography alone or guided by FFR measurements in addition to angiography^[81]. Patients assigned to angiography-guided PCI underwent stenting of all indicated lesions, whereas those assigned to FFR-guided PCI underwent stenting of all indicated lesions only if the FFR was 0.80 or less. The primary endpoint was the rate of death, nonfatal myocardial infarction, and repeat re-

vascularization at 1 year. The number of indicated lesions per patient was 2.7 ± 0.9 in the angiography group and 2.8 ± 1.0 in the FFR group ($P = 0.34$). The number of stents used per patient was 2.7 ± 1.2 and 1.9 ± 1.3 , respectively ($P < 0.001$). The 1-year event rate was 18.3% in the angiography group and 13.2% in the FFR group ($P = 0.02$). The rate of death and myocardial infarction was 11.1% in the angiography group and 7.3% in the FFR group ($P = 0.04$). Pijls *et al*^[82] reported the 2-year follow-up results of the FAME study. The 2-year rates of mortality or myocardial infarction were 12.9% in the angiography-guided group and 8.4% in the FFR-guided group ($P = 0.02$). Combined rates of death, nonfatal myocardial infarction, and revascularization were 22.4% and 17.9%, respectively ($P = 0.08$). For lesions deferred on the basis of FFR > 0.80 , the rate of myocardial infarction was 0.2% and the rate of revascularization was 3.2% after 2 years, which is a very low rate. Thus, routine measurement of FFR in patients with multivessel coronary artery disease who undergo PCI with drug-eluting stents significantly reduced the rate of death, nonfatal myocardial infarction, and repeat revascularization for up to 2 years.

Tonino *et al*^[83] studied the angiographic *vs* functional severity of coronary artery stenosis in the FAME study. Of the 1414 lesions (509 patients) in the FFR-guided arm of the FAME study, 1329 were successfully assessed by the FFR. Before FFR measurement, these lesions were categorized into 50%-70%, 71%-90%, and 91%-99% diameter stenosis by visual assessment. In the category 50%-70% stenosis, only 35% were functionally significant. In the category 71%-90% stenosis, 80% were functionally significant and in the category of subtotal stenoses, 96% were functionally significant. Of all 509 patients with angiographically defined multivessel disease, only 235 (46%) had functional multivessel disease.

In FAME 2 study, investigators enrolled patients with stable coronary artery disease for whom PCI was being considered, and assessed all stenoses by measuring FFR^[84]. Patients in whom at least one stenosis was functionally significant (FFR ≤ 0.80) were randomly assigned to FFR-guided PCI plus the best available MT (PCI group), or the best available MT alone (MT group). Patients in whom all stenoses had an FFR of more than 0.80 were entered into a registry and received the best available MT. The primary endpoint was a composite of death, myocardial infarction, or urgent revascularization. Recruitment was halted prematurely after enrollment of 1220 patients (888 who underwent randomization and 332 enrolled in the registry) because of a significant between-group difference in the percentage of patients who had a primary endpoint event: 4.3% in the PCI group and 12.7% in the MT group (HR with PCI: 0.32; 95%CI: 0.19-0.53; $P < 0.001$). The difference was driven by a lower rate of urgent revascularization in the PCI group than in the MT group (1.6% *vs* 11.1%; HR = 0.13; 95%CI: 0.06-0.30; $P < 0.001$). Among patients in the registry, 3.0% had a primary endpoint event, which was not significantly different from the PCI group. Thus, in

patients with stable coronary artery disease and functionally significant stenoses, FFR-guided PCI plus the best available MT, as compared with the best available MT alone, decreased the need for urgent revascularization. In patients without ischemia, the outcome appeared to be favorable with the best available MT alone. The main reason why there was no significant difference in death and myocardial infarction between the PCI group and MT group seems to be the relatively small number of patients and short-term follow-up period (mean duration of follow-up was 213 ± 128 d in the PCI group and 214 ± 127 d in the MT group).

Pijls *et al*^[80] explain why FFR-guided PCI decreases the rate of death and myocardial infarction in the FAME study. From many studies it is known that the death and myocardial infarction rates are less than 1% per year for a functionally nonsignificant stenosis if treated appropriately by medication, between 5% and 10% per year for a functionally significant stenosis if only treated by medication, and approximately 3% per year for a stented lesion whether it was functionally significant or not. Thus, stenting a functionally significant stenosis improves outcome, but stenting a functionally nonsignificant stenosis worsens outcome. Taken together, these studies suggest that IG PCI is superior to angiography-guided PCI, and the presence of ischemia is the key to the decision-making for PCI.

APPROPRIATENESS CRITERIA

For many years, the American College of Cardiology (ACC) and American Heart Association (AHA) have jointly published and updated guidelines for PCI and CABG^[85,86]. Recently, the ACC Foundation/Society for Cardiovascular Angiography and Interventions/Society for Thoracic Surgeons/American Association for Thoracic Surgery/AHA/American Society of Nuclear Radiology released appropriateness criteria for coronary revascularization to serve as a supplement to the ACC/AHA guideline documents^[87].

Hannan *et al*^[88] studied the appropriateness of PCI and CABG performed in New York for patients without acute coronary syndrome or previous CABG. Of the 8168 patients undergoing CABG, 90.0% were appropriate for revascularization, 1.1% were inappropriate, and 8.6% were uncertain. Of the 33970 PCI patients, 28% lacked sufficient information to be rated. Of the patients who could be rated, 36.1% were appropriate, 14.3% were inappropriate, and 49.6% were uncertain. A total of 91% of the patients undergoing PCI who were classified as inappropriate had one- or two-vessel disease without proximal left anterior descending artery disease, and had no or minimal anti-ischemic MT. Chan *et al*^[89] studied 500154 patients enrolled in the National Cardiovascular Data Registry. For 355417 patients with acute indications, 98.6% were classified as appropriate, 1.1% as inappropriate, and 0.3% as uncertain. For 144737 patients with nonacute indications, 50.4% were classified as appropri-

ate, 11.6% as inappropriate, and 38.0% as uncertain. The majority of inappropriate PCIs for nonacute indications were performed in patients with no angina (53.8%), low-risk ischemia on noninvasive stress testing (71.6%), or suboptimal (≤ 1 medication) antianginal therapy (95.8%). Furthermore, although variation in the proportion of inappropriate PCI across hospitals was minimal for acute procedures, there was substantial hospital variation for nonacute procedures (mean hospital rate for inappropriate PCI, 10.8%; interquartile range, 6.0%-16.7%).

Lin *et al*^[90] studied the frequency and predictors of stress testing prior to elective PCI in a Medicare population of 23887 patients. Only 44.5% of patients underwent stress testing within 90 d prior to elective PCI. There were wide regional variations among the hospital referral regions, with stress testing ranging from 22.1% to 70.6% (mean, 44.5%, interquartile range 39.0%-50.9%). Female sex [adjusted OR (aOR) = 0.91; 95%CI: 0.86-0.97], age 85 years or older (aOR = 0.83; 95%CI: 0.72-0.95), a history of congestive heart failure (aOR = 0.85; 95%CI: 0.79-0.92), and prior cardiac catheterization (aOR = 0.45; 95%CI: 0.38-0.54) were associated with a decreased likelihood of prior stress testing. Thus, these studies demonstrated that, although PCI in the acute setting and CABG are properly performed in most patients, PCI in the nonacute setting is often inappropriate, and stress testing to identify myocardial ischemia is performed in less than half of patients.

Some studies also showed that revascularization in an inappropriate setting is not associated with improved prognosis. Ko *et al*^[91] assessed the appropriateness of coronary revascularization (PCI or CABG) and examined its association with longer-term outcomes. In 1625 patients with stable coronary artery disease, coronary revascularization was performed in only 69% in the appropriate category, 45% in the inappropriate category, and 54% in the uncertain category. In patients in the appropriate category, coronary revascularization was associated with a lower adjusted hazard of death or acute coronary syndrome (aHR = 0.61; 95%CI: 0.42-0.88; $P = 0.0087$) at 3 years compared with MT. No significant differences in death or acute coronary syndrome were observed between coronary revascularization and MT in the inappropriate category (aHR = 0.99; 95%CI: 0.48-2.02) and the uncertain category (aHR = 0.57; 95%CI: 0.28-1.16; $P = 0.12$).

FUTURE PERSPECTIVE

Both MPI and FFR clearly identify the presence or absence of myocardial ischemia, and IG revascularization is associated with improved prognosis. However, the FFR value which is concordant with a 10% ischemic myocardium by MPI remains to be determined. A cut-off value of 0.75 was determined by the positive or negative results of three noninvasive stress tests; bicycle exercise test, thallium scintigraphy, and stress echocardiography with dobutamine^[92]. A FFR value between

0.75 and 0.80 is deemed to be in the gray zone. MPI has limitation in identification of the highest risk subsets, left main coronary artery disease and three-vessel coronary artery disease, because of “balanced ischemia”^[93-98]. One study showed that in patients with left main coronary artery disease, MPI results were normal in 5% and low-risk in 10% of patients^[93]. The other study showed that in patients with triple-vessel coronary artery disease, MPI results were normal in 12% and single-vessel in 28% of patients^[94].

Some studies compared MPI and FFR in patients with multivessel coronary artery disease. Ragosta *et al*^[99] performed angiography, FFR, and MPI in 36 patients (88 arteries), and determined the association between FFR and perfusion for each vascular zone. Concordance between angiography, FFR, and MPI was seen in 61 of 88 zones (69%). Discordance was seen in the remaining 27 zones (31%), and was predominantly related to the finding of a FFR < 0.75 or total occlusion despite no defect on MPI. Melikian *et al*^[100] performed MPI and FFR in 67 patients (201 vessels) with angiographic two- or three-vessel coronary artery disease. In 42% of patients, MPI and FFR detected identical ischemic areas (mean number of areas 0.9 ± 0.8 for both, $P = 1.00$). In the remaining 36% MPI underestimated the number (MPI = 0.46 ± 0.6 , FFR = 2.0 ± 0.6 , $P < 0.001$) and in 22% overestimated the number (MPI = 1.9 ± 0.8 , FFR = 0.5 ± 0.8 , $P < 0.001$) in comparison with FFR. Thus, MPI has poor concordance with FFR and tends to underestimate or overestimate the functional importance of coronary stenosis in comparison with FFR in patients with multivessel disease. In patients with multivessel coronary artery disease, FFR is the preferred method to identify myocardial ischemia. Therefore, complementary use of noninvasive MPI and invasive FFR would be important to compensate for each method's limitations.

CONCLUSION

MPI studies demonstrate that for patients with moderate to severe ischemia, revascularization is the preferred therapy for survival benefit. For patients with no to mild ischemia, MT is the main choice and revascularization is associated with increased mortality probably because of worsened ischemia. FFR studies demonstrate that IG PCI is superior to angiography-guided PCI, and the presence of ischemia is the key to decision-making for PCI. Studies of appropriateness criteria demonstrate that, although CABG and emergency PCI are appropriately performed in most patients, use of elective PCI is often inappropriate. Some studies also suggest that revascularization in an inappropriate setting is not associated with improved prognosis. Taken together, myocardial ischemia is a key factor in the management of patients with stable coronary artery disease.

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WJC 6th Anniversary Special Issues (5): Myocardial infarction

Clinical significance of glycated hemoglobin in the acute phase of ST elevation myocardial infarction

Chiara Lazzeri, Serafina Valente, Marco Chiostrì, Maria Grazia D'Alfonso, Gian Franco Gensini

Chiara Lazzeri, Serafina Valente, Marco Chiostrì, Maria Grazia D'Alfonso, Gian Franco Gensini, Intensive Cardiac Care Unit, Heart and Vessel Department, Azienda Ospedaliero, Universitaria Careggi, 50134 Florence, Italy

Author contributions: Lazzeri C and Valente S designed the study and wrote the manuscript; Chiostrì M and D'Alfonso MG collected the data; Gensini GF designed the study.

Correspondence to: Chiara Lazzeri, MD, Intensive Cardiac Care Unit, Heart and Vessel Department, Azienda Ospedaliero, Universitaria Careggi, Viale Morgagni 85, 50134 Florence, Italy. lazzeric@libero.it

Telephone: +39-55-7947518 Fax: +39-55-7947518

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has several advantages over fasting plasma glucose or an oral glucose tolerance test in an acute setting. The test can be performed in the non-fasting state and reflects average glucose concentration over the preceding 2-3 mo. We therefore proposed an algorithm based on pragmatic grounds which could be applied in STEMI patients without known diabetes in order to detect glucose intolerance abnormalities from the early phase. The main advantage of this algorithm is that it may help in tailoring the follow-up program, by helping in identifying patients at risk for the development of glucose intolerance after MI. Further validation of this algorithm in prospective studies may be required in the contemporary STEMI population to resolve some of these uncertainties around HbA1c screening cutoff points.

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Key words: Glycated hemoglobin; ST-elevation myocardial infarction; Prognosis; Hyperglycemia; Glucose intolerance

Abstract

In population-based studies, including diabetic and nondiabetic cohorts, glycated hemoglobin A1c (HbA1c) has been reported as an independent predictor of all-cause and cardiovascular disease mortality. Data on the prognostic role of HbA1c in patients with acute myocardial infarction (MI) are not univocal since they stem from studies which mainly differ in patients' selection criteria, therapy (thrombolysis *vs* mechanical revascularization) and number consistency. The present review is focused on available evidence on the prognostic significance of HbA1c measured in the acute phase in patients with ST-elevation myocardial infarction (STEMI) submitted to primary percutaneous coronary intervention (PCI). We furthermore highlighted the role of HbA1c as a screening tool for glucose intolerance in patients with STEMI. According to available evidence, in contemporary cohorts of STEMI patients submitted to mechanical revascularization, HbA1c does not seem to be associated with short and long term mortality rates. However, HbA1c may represent a screening tool for glucose intolerance from the early phase on in STEMI patients. On a pragmatic ground, an HbA1c test

Core tip: Data on the prognostic role of glycated hemoglobin A1c (HbA1c) in patients with acute myocardial infarction (MI) are not univocal since they stem from studies which mainly differ in patients' selection criteria, therapy (thrombolysis *vs* mechanical revascularization) and number consistency. According to available evidence, in contemporary cohorts of ST-elevation myocardial infarction (STEMI) patients submitted to mechanical revascularization, HbA1c does not seem to be associated with short and long term mortality. However, in STEMI patients, HbA1c, even measured in the early phase, may represent a screening tool for glucose intolerance since its measurement can be performed in the non-fasting state and reflects average glucose concentration over the preceding 2-3 mo. We therefore proposed an algorithm based on pragmatic grounds

which could be applied in STEMI patients without known diabetes in order to detect glucose intolerance abnormalities from the early phase. The main advantage of this algorithm is that it may help in tailoring the follow-up program, by helping in identifying patients at risk for the development of glucose intolerance after MI.

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INTRODUCTION

Discovered more than forty years ago by Rahbar *et al*^[1], the breakthrough for glycated hemoglobin A1c (HbA1c) was achieved when it was discovered in the Diabetes Control and Complications Trial in 1993 that the concentration of HbA1c was an excellent predictor of diabetes-related long-term complications^[2].

In population-based studies^[3], including diabetic and nondiabetic cohorts, HbA1c has been reported as an independent predictor of all-cause and cardiovascular disease (CDV) mortality^[4-6]. Among individuals with diabetes, every 1% rise in HbA1c is associated with a 30% increase in all-cause mortality and a 40% increase in CVD mortality^[7]. In the Reykjavik Study and in a meta-analysis of other Western prospective studies, fasting and post-load glucose levels were modestly associated with coronary heart disease (CHD) risk in people without diabetes^[8], while associations of HbA1c with CHD risk in such people appeared somewhat stronger (a RR for CHD of 1.20 per 1% higher HbA1c). In a community-based population study, elevated HbA1c has been recently reported to be predictive for CDV and mortality in patients without diabetes mellitus, regardless of fasting glucose levels^[9].

Data on the prognostic role of HbA1c in patients with acute myocardial infarction (AMI) stem from studies which mainly differ for patients' selection criteria, therapy (thrombolysis *vs* mechanical revascularization) and number consistency.

The present review is focused on available evidence on the prognostic significance of HbA1c measured in the acute phase in patients with ST-elevation myocardial infarction (STEMI) submitted to primary percutaneous coronary intervention (PCI). We furthermore highlighted the role of HbA1c as a screening tool for glucose intolerance in these patients.

GLYCATED HEMOGLOBIN AS A PROGNOSTIC TOOL IN STEMI PATIENTS

Glycated hemoglobin and patients without known diabetes and with ST elevation myocardial infarction

Only small studies assessed the prognostic role of HbA1c

in STEMI patients without a history of diabetes and results are not univocal due to differences in patients' selection criteria and methods^[10-13]. In 150 non diabetic patients with myocardial infarction (MI), mortality rate and the risk of cardiogenic shock increased with HbA1c^[10]. In a high-risk MI population^[12], HbA1c was a risk marker of death at follow-up in patients without a history of diabetes and not in diabetic patients, while, in a small group of MI patients (diabetic and not diabetic) treated with thrombolysis^[11], there were significant relationships between admission glucose, HbA1c level and mortality at follow-up. Similarly, in 374 STEMI patients (diabetic and not diabetic), after adjusting for baseline characteristics, HbA1c remained a strong independent predictor of in-hospital mortality (OR = 1.412; 95%CI: 1.031-1.935, $P = 0.03$)^[14].

On the other hand, in 504 unselected, consecutive non diabetic STEMI patients submitted to PCI, hyperglycemia (not glycated hemoglobin) was a predictor of 30-d outcome^[13]. We recently^[15] assessed the prognostic role of HbA1c for mortality at short and long terms in 518 consecutive STEMI patients without previously known diabetes, all submitted to mechanical revascularization. Patients with HbA1c $\geq 6.5\%$ showed higher values of admission, peak and discharge glucose ($P < 0.001$, $P < 0.001$ and $P < 0.001$, respectively) and a higher incidence of acute insulin resistance [as inferred by the Homeostatic Model Assessment index (HOMA)] ($P = 0.001$) as well as higher values of fibrinogen ($P < 0.001$) and triglycerides ($P = 0.001$) and lower values of HDL ($P = 0.018$). No differences in short and long-term mortality rates and in the use of devices were detectable between patients with HbA1c $< 6.5\%$ and those with HbA1c $\geq 6.5\%$. At multivariate backward logistic regression analysis HbA1c was not associated with in-hospital death (OR = 7.210, 95%CI: 0.75-69.69, $P = 0.088$). At follow-up [median 39.7 (22.2-57.1) mo], a Kaplan-Meier survival curve documented no significant differences between patients with HbA1c $< 6.5\%$ and those with HbA1c $\geq 6.5\%$. In our study population, patients with HbA1c levels higher than 6.5% did not show a higher infarct size (as indicated by TnI and left ventricular ejection fraction) or a more critical illness (as inferred by the use of devices). Discrepancies with previous papers are mainly related to number consistency^[10], population selection criteria^[11] and type of revascularization^[13]. As a difference from previous studies^[10,11,13], we observed that higher HbA1c values help in identifying a subset of patients who, in the early phase of STEMI, show an abnormal glucose response to stress as indicated by higher values of glucose, worse glycemic control during Intensive Cardiac Care Union (ICCU) stay (peak glycemia) and a higher incidence of acute insulin resistance (HOMA index). All these factors have been associated with increased risk of early death as reported by Deedwania *et al*^[16] and by us in previous reports^[17-21]. Patients with HbA1c $> 6.5\%$ also showed an increased inflammatory activation (increased values of fibrinogen), suggesting a link between acute glucose

dysmetabolism and inflammatory activation in the early phase of STEMI^[16].

Similar results were recently reported by Tian *et al.*^[22] in an observational multicenter study performed in 608 STEMI patients submitted to primary PCI. The study population was stratified according to the new American Diabetes Association criteria, into three groups: I, HbA1c 5.6% or less ($n = 262$); II, HbA1c 5.7%-6.4% ($n = 182$); and III, HbA1c at least 6.5% ($n = 164$). The 7-d mortality was similar ($P = 0.179$) between groups I (1.9%), II (2.2%), and III (0.0%) as well as the 30-d mortality ($P = 0.241$) between groups I (3.8%), II (2.2%), and III (1.2%). Major adverse cardiac events at the 7-d and 30-d follow-up were not significantly different between the three groups either ($P > 0.05$). After adjusting the baseline characteristics, HbA1c was not an independent predictor of short-term outcomes (HR = 0.431; 95%CI: 0.175-1.061, $P = 0.067$).

Glycated hemoglobin and patients with known diabetes and with STEMI

In patients with AMI and diabetes, the two Diabetes Insulin Glucose in AMI studies both showed that increasing HbA1c levels increased mortality in diabetic patients with MI^[23,24]. Conversely^[12], in Optimal Trial in Myocardial Infarction with the Angiotensin II Antagonist Losartan trial (including patients with MI complicated by heart failure) the level of HbA1c had no impact on mortality among the patients with well-known diabetes. Similarly, in consecutive diabetic patients undergoing PCI^[25], HbA1c was not a predictor of cardiac events at one-year follow-up.

In a recent investigation^[26], which includes the largest series of consecutive STEMI patients with known diabetes submitted to mechanical revascularization, we observed that HbA1c was not associated with mortality in either the short or the long term. Nevertheless, higher HbA1c values (which were detectable in about half of the entire population) helped to identify a subset of patients who, in the early phase of STEMI, showed an abnormal glucose response to stress as indicated by higher values of glucose, a worse glycemic control during ICCU stay (as inferred by peak glycemia) and a higher incidence of acute insulin resistance (as indicated by HOMA index). This subset of patients may deserve a more aggressive treatment for glucose management, since previous studies performed by other investigators^[16] and by us^[17-21,26,27] showed that admission glycemia and peak glycemia are independent predictors for in-hospital mortality in STEMI patients.

Glycated hemoglobin and long term mortality in STEMI patients

In the thrombolytic era, in two small studies both excluding patients with newly diagnosed diabetes^[28,29], an independent effect on mortality of HbA1c was reported in nondiabetic patients with MI. HbA1c levels higher than 6.5% were associated with higher ischemic score

in patients with MI (diabetic and non diabetic) submitted to thrombolysis^[13], and significant relationships were observed between admission glucose, HbA1c level and mortality at follow-up. Glycated hemoglobin was a potent risk marker of death at follow-up only in MI patients without a history of diabetes but not in diabetic patients^[12]. Conversely, elevated admission glucose (and not glycated hemoglobin) was an important predictor of 30-d outcome after STEMI in 504 unselected, consecutive non diabetic patients with STEMI submitted to PCI^[11]. Chan *et al.*^[30] reported, in a small cohort of 317 diabetic patients with acute coronary syndrome, that HbA1c levels before admission were not associated with short-term cardiovascular outcome (all-cause mortality, cardiovascular mortality, symptom driven revascularization, rehospitalization for angina, and hospitalization for heart failure).

On the other hand, Timmer *et al.*^[31] observed that increasing quartiles of HbA1c (even below the diagnostic threshold for diabetes mellitus) were associated with increased mortality rates over an average 3.3 years of follow-up in 4176 consecutive STEMI patients without known diabetes submitted to PCI. This finding was partially related to the fact that increasing HbA1c levels were associated with adverse baseline characteristics such as a higher cardiovascular risk profile.

In a large contemporary cohort of 1205 consecutive patients with STEMI submitted to PCI, we recently^[32] assessed the impact of increased HbA1c ($\geq 6.5\%$) on long term mortality. In our series 276 patients with previously diagnosed diabetes (276/1205, 22.9%, Group A), 78 patients without previously known diabetes and HbA1c $\geq 6.5\%$ (78/1205, 6.5%, Group B) and 851 patients without previously known diabetes and HbA1c $< 6.5\%$ (851/1205, 70.1%, Group C). At Cox regression analysis, HbA1c $\geq 6.5\%$ was not related to 1-year post discharge mortality in patients with previously diagnosed diabetes (Group A) nor in those without previously known diabetes (Group B and C). Kaplan-Meier survival curve analysis showed that patients in Group A exhibited the lowest survival rate, while patients in Group B (that is patients without previously known diabetes and with HbA1c $\geq 6.5\%$) showed a significant reduction in their survival rate since 6-mo after discharge. In conclusion, in our investigation HbA1c levels were not related with outcomes at multivariable analysis in a large cohort of unselected STEMI patients submitted to PCI.

GLYCATED HEMOGLOBIN AS A SCREENING TOOL FOR GLUCOSE INTOLERANCE IN STEMI PATIENTS IN THE ACUTE PHASE

More than 18 million people in the United States have diabetes mellitus, and approximately 35% of the population is prediabetic^[33]. Another 7 million Americans have undiagnosed diabetes and are at high risk of developing

Table 1 Prevalence of glucose intolerance in patients with acute myocardial infarction

Ref.	Patients	Methods	Prevalence	Results
Norhammar <i>et al</i> ^[40] , 2002	81 non diabetic AMI patients	OGTT	Diabetes: 31% IGT: 35%	HbA1c on admission was independent predictor of glucose intolerance at 3 mo ($P = 0.024$)
Ishihara <i>et al</i> ^[53] , 2006	200 non diabetic patients with AMI	OGTT	Diabetes: 27%	Fasting glucose and HbA1c were independent predictors of abnormal glucose tolerance, but admission glucose was not.
Gustafsson <i>et al</i> ^[12] , 2007	2841 patients with heart failure complicating AMI	HbA1c	History of diabetes: 17% HbA1c < 4.9%: 58% HbA1c 4.9%-5.1%: 15% HbA1c > 5.1%: 10%	In non diabetic patients, a 1% absolute increase in HbA1c level at baseline resulted in a 24% increase in mortality In diabetic patients, the level of HbA1c had no impact on mortality
Rasoul <i>et al</i> ^[11] , 2007	504 non diabetic STEMI	HbA1c	HbA1c < 6.0%: 82.5% HbA1c > 6.0%: 17.5%	HbA1c was not associated with 30-d mortality
Cakmak <i>et al</i> ^[13] , 2008	100 non diabetic patients with AMI treated with thrombolysis; patients on antidiabetic therapy excluded	HbA1c	HbA1c 4.5-6.4%: 25% HbA1c 6.5-8.5%: 28% HbA1c > 8.5%: 47%	Admission HbA1c was significantly correlated with mortality ($P = 0.009$)
Knudsen <i>et al</i> ^[47] , 2009	224 non diabetic STEMI	OGTT	Abnormal glucose regulation: 46.9% in the early phase 24.9% at 3 mo	High levels of HbA1c and admission plasma glucose in-hospital significantly predicted abnormal glucose regulation at 3 mo ($P < 0.001$)
Timmer <i>et al</i> ^[31] , 2011	4176 non diabetic STEMI patients	HbA1c quartiles	IQR1 $\leq 5.35\%$: 27% IQR2 5.6%-5.54%: 24% IQR3 5.55%-5.80%: 25% IQR4 $\geq 5.81\%$: 24%	HbA1c (hazard ratio, 1.2 per interquartile range; $P < 0.01$), but not glucose, was independently associated with long-term mortality
Lazzeri <i>et al</i> ^[15] , 2012	518 non diabetic STEMI patients	HbA1c	HbA1c < 6.5%: 90.4% HbA1c $\geq 6.5\%$: 9.6%	HbA1c was not associated with short and long term mortality
Tian <i>et al</i> ^[22] , 2013	608 STEMI	Hb1c groups	I: HbA1c $\leq 5.6\%$: 43% II: HbA1c 5.7%-6.4%: 30% III: HbA1c $\geq 6.5\%$: 27%	After adjusting the baseline characteristics, HbA1c was not an independent predictor of short-term outcomes (HR = 0.431; 95%CI: 0.175-1.061, $P = 0.067$)
Lazzeri <i>et al</i> ^[32] , 2013	1204 STEMI patients	HbA1c	Diabetic patients: 22.9% patients without known diabetes: HbA1c < 6.5%: 70.1% HbA1c $\geq 6.5\%$: 6.5%	At Cox regression analysis, HbA1c $\geq 6.5\%$ was not related to 1-yr post discharge mortality in diabetic and in non diabetic patients

HbA1c: Glycated hemoglobin A1c; OGTT: Oral glucose tolerance test; AMI: Acute myocardial infarction; IGT: Impaired glucose tolerance; STEMI: ST-elevation myocardial infarction; IQR: Interquartile range.

diabetic complications, including CDV^[34,35]. These numbers are expected to continue to rise in the United States and worldwide in large part due to the growing obesity epidemic^[36-38]. In 2010, an estimated 6.4% of the world's adult population (approximately 285 million individuals) had diabetes, and the prevalence is projected to increase to 7.7% (approximately 439 million individuals) by 2030^[39].

Prevalence of glucose intolerance in STEMI patients

In the glucose tolerance in AMI study^[40], HbA1c independently predicted glucose intolerance (OR = 2.58 95%CI: 1.17-6.09, $P = 0.024$) in people with acute coronary syndrome without known diabetes, correlating closely with the 2-h plasma glucose in an oral glucose tolerance test ($r = 0.39$, $P < 0.0001$). Furthermore, an HbA1c ≥ 30 mmol/mol (4.9%) had sensitivity and specificity of 79% and 49% for detecting undiagnosed diabetes, respectively, with the area under curve of 0.685 ($P = 0.001$). In the Euro Heart Survey on diabetes, 22% of people admitted to hospital as emergency cases because of coronary artery disease were found to have undiagnosed diabetes after a glucose tolerance test, with a further 36% found to have impaired glucose tolerance^[41].

It has been recently observed among patients with high-risk non-ST-segment elevation acute coronary syndrome (NSTEMI ACS)^[42] that a substantial proportion of patients admitted with high-risk NSTEMI ACS had previously undiagnosed diabetes mellitus (12.2%) or prediabetes (10.8%) as defined by fasting glucose or HbA1c after hospital admission.

Table 1 shows the prevalence of glucose intolerance according to existing investigations on this topic in patients with AMI. These studies were selected by a PubMed search matching "acute myocardial infarction/STEMI/acute coronary syndrome" and "glucose intolerance/hyperglycemia/glycated hemoglobin".

The prevalence of STEMI patients with glucose intolerance, as detected mainly by HbA1c measured in the early phase, varies, ranging from 10% to more than 40%. Differences can be mainly related to the chosen value of HbA1c. More recently, in an observational multicenter study, Tian *et al*^[22] stratified the study population according to HbA1c values and observed that the percentage of patients with HbA1c > 5.7% accounted for more than 50%.

On a clinical ground, in STEMI patients, early diag-

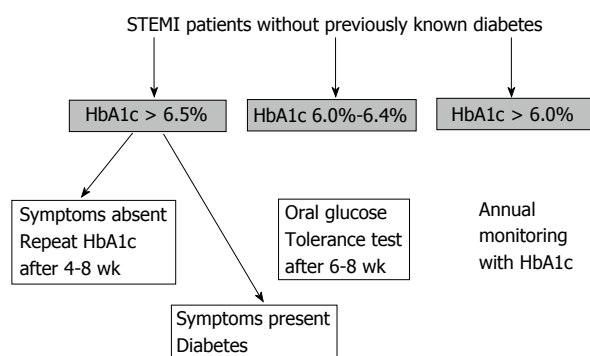


Figure 1 A screening algorithm for glucose intolerance based on glycated hemoglobin. STEMI: ST-elevation myocardial infarction; HbA1c: Glycated hemoglobin A1c.

nosis of unknown type 2 diabetes or impaired glucose regulation allows initiation of treatment or lifestyle interventions, including diet and exercise to prevent type 2 diabetes and associated complications. Gaining information on family history for diabetes could help in identifying subjects with undiagnosed diabetes or at risk^[43,44].

However, in the acute phase of STEMI, the identification of glucose intolerance is quite difficult since the common finding of hyperglycemia, irrespective of underlying diabetic status, is to be related mainly to the acute stress response^[16-21,26] to myocardial ischemia^[45].

Strategy for screening for glucose intolerance in STEMI patients according to glycated hemoglobin

Recently, National Institute for Health and Clinical Excellence (NICE) guidelines on the management of hyperglycaemia in acute coronary syndrome have advocated any hyperglycaemia (blood glucose > 11.0 mmol/L) without known diabetes be followed up with an HbA1c measurement before discharge and fasting plasma glucose test 4 d after the onset of acute coronary syndrome^[46]. NICE recommend against routine use of the oral glucose tolerance test in patients with acute coronary syndrome and with fasting plasma glucose and HbA1c in the normal range. However, guidance on categorization of glycaemic status of those with elevated HbA1c and fasting plasma glucose, as well as screening for diabetes in those without hyperglycaemia, is less clear. As a consequence, the lack of simple strategy for early identification of glucose intolerance in acute coronary syndrome is potentially leaving many people undiagnosed and under-treated, especially after the cardiac event.

The oral glucose tolerance test is performed infrequently in the acute setting^[41], since it is time consuming, not always well tolerated and it does not seem to provide reliable information on long-term glucometabolic state^[47].

In the early phase of STEMI, fasting plasma glucose can be acutely elevated and therefore unreliable in the first 2 d of an acute event and in a large MI^[48]. NICE has suggested fasting plasma glucose testing should not be conducted within the first 4 d of the acute event. Howev-

er, in the current era of early reperfusion therapies, many patients with acute coronary syndrome are discharged earlier.

On a pragmatic ground, an HbA1c test has several advantages over fasting plasma glucose or an oral glucose tolerance test in an acute setting. The test can be performed in the non-fasting state and reflects average glucose concentration over the preceding 2-3 mo. Therefore, in our opinion, glycated hemoglobin should be measured in all patients with STEMI.

Measuring HbA1c assumes International Federation of Clinical Chemistry standardized laboratory assays are used. Furthermore, conditions precluding accurate measurement of HbA1c concentration for diagnosis should be excluded, including abnormalities of red cell turnover, chronic renal or liver failure and chronic use of certain medications.

We therefore proposed an algorithm (Figure 1) based on pragmatic grounds (and our experience) which should be applied in STEMI patients without known diabetes in order to detect glucose intolerance abnormalities since the early phase.

Above HbA1c > 6.5%, individuals should be assessed for symptoms of diabetes (*i.e.*, increased thirst, polyuria, unexplained weight loss, blurred vision, extreme fatigue), ruling out other causes, for example polyuria attributable to diuretic therapy. In those with unequivocal symptoms the diagnosis is confirmed^[49]. Conversely, those with ambiguous or absent symptoms should undergo a confirmatory HbA1c measurement 4-8 wk post-discharge for consistency and to counteract any potential laboratory errors on the first occasion.

Patients with HbA1c between 6.0% and < 6.4% should undergo an oral glucose tolerance test after 6-8 wk.

STEMI patients without known diabetes and HbA1c < 6.0% should undergo annual surveillance with HbA1c as incident impaired glucose regulation and diabetes is higher compared with the general population^[50].

The main advantage of this algorithm is that it may help in tailoring the follow-up program, by helping to identify patients at risk for the development of glucose intolerance after MI.

Further validation of this algorithm in prospective studies may be required in the contemporary STEMI population to resolve some of these uncertainties around HbA1c screening cut points.

Given the increasing focus on managing multiple co-existing illnesses affecting cardiovascular patients^[51], the assessment of glycosylated hemoglobin (HbA1c) in patients with STEMI could be an important opportunity to improve care for these patients^[52].

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Duration of dual antiplatelet treatment in the era of next generation drug-eluting stents

Seung-Woon Rha

Seung-Woon Rha, Cardiovascular Center, Korea University Guro Hospital, Seoul 152-703, South Korea

Author contributions: Rha SW solely contributed to this paper.
Correspondence to: Seung-Woon Rha, MD, PhD, FACC, FAHA, FESC, FSCAI, Cardiovascular Center, Korea University Guro Hospital, 80, Guro-dong, Guro-gu, Seoul 152-703, South Korea. swrha617@yahoo.co.kr

Telephone: +82-2-26263020 Fax: +82-2-8643062

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Abstract

Current percutaneous coronary intervention guidelines recommend dual antiplatelets (aspirin 100 mg + clopidogrel 75 mg daily) for at least 12 mo following drug-eluting stent (DES) implantation if patients are not at high risk of bleeding. Several reports have tried to shorten the dual antiplatelet therapy to 3-6 mo, especially following next-generation DES implantation, for cost-effectiveness. However, the clinical results are inconsistent and the data regarding next-generation DESs limited. In this report, recently published important pivotal reports regarding the optimal duration of dual antiplatelets following DES implantation are summarized.

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Key words: Drug-eluting stent; Dual antiplatelet treatment; Percutaneous coronary intervention

Core tip: Recently published important pivotal reports regarding the optimal duration of dual antiplatelets following drug-eluting stent implantation are summarized.

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INTRODUCTION

Multiple randomized clinical trials have shown the efficacy of drug-eluting stents (DES) in reducing restenosis and the need for target lesion revascularization (TLR) compared with bare-metal stents (BMS)^[1,2]. Despite the reduced incidence of recurrence, safety issues related to DESs, such as stent thrombosis, late stent malapposition, aneurysm, stent fracture, endothelial dysfunction and restenosis, have been reported elsewhere, particularly with first-generation DESs. Furthermore, some observational studies have shown that the risk of death or myocardial infarction was even higher with DESs than BMSs, possibly due to a higher incidence of late or very late stent thrombosis^[3].

Early or premature discontinuation of dual antiplatelet therapy has been reported as an important risk factor for late stent thrombosis following DES implantation^[4,5]. Thus, current percutaneous coronary intervention (PCI) guidelines recommend dual antiplatelets (aspirin + clopidogrel 75 mg daily) for at least 12 mo following DES implantation if patients are not at high risk of bleeding^[6]. Several reports have tried to address this issue but the results are inconsistent and the data regarding second-generation DESs limited. In this report, the important pivotal reports regarding the optimal duration of dual antiplatelets following DES implantation, particularly in patients who underwent PCI with next generation DESs, are summarized.

OPTIMAL DURATION OF DUAL ANTIPLATELET THERAPY WITH DESs

Major clinical trials for duration of dual antiplatelets after DES implantation

REAL-LATE and ZEST-LATE trial: (Aspirin +

Table 1 Clinical outcomes at 12 mo and 24 mo¹

Clinical outcomes	At 12 mo		At 24 mo		HR (95%CI) ²		P
	Clopidogrel + aspirin	Aspirin alone	Clopidogrel + aspirin	Aspirin alone	Clopidogrel + aspirin	Aspirin alone	
Primary end point: MI or death from cardiac causes	0.7	0.5	1.8	1.2	1.65 (0.80-3.36)		0.17
Secondary end points							
Death from any cause	0.5	0.5	1.6	1.4	1.52 (0.75-3.50)		0.24
MI	0.4	0.3	0.8	0.7	1.41 (0.54-3.71)		0.49
Stroke	0.3	0.3	1.0	0.3	2.22 (0.68-7.20)		0.19
Stent thrombosis, definite	0.2	0.1	0.4	0.4	1.23 (0.33-4.58)		0.76
Repeat revascularization	1.7	1.1	3.1	2.4	1.37 (0.83-2.27)		0.22
MI or death from any cause	0.8	0.8	2.3	1.7	1.57 (0.85-2.88)		0.15
MI, stroke, or death from any cause	1.1	1.1	3.2	1.8	1.73 (0.99-3.00)		0.05
MI, stroke, or death from cardiac causes	1.0	0.8	2.7	1.3	1.84 (0.99-3.45)		0.06
Major bleeding, according to TIMI criteria	0.2	0.1	0.2	0.1	2.96 (0.31-28.46)		0.35

¹For the total number of events for each type of end point, only the first event is counted. Cumulative rates of events are based on Kaplan-Meier estimates. All deaths were considered to be from cardiac causes unless an unequivocal noncardiac cause could be established; ²Hazard ratios are for the dual-therapy group as compared with the aspirin-alone group. MI: Myocardial infarction; TIMI: Thrombolysis in myocardial infarction. (Modified from Ref. [7]).

clopidogrel *vs* aspirin alone after 1 year). A randomized trial from South Korea showed that dual antiplatelets for longer than 12 mo following DES implantation was not significantly more effective than aspirin monotherapy^[7]. In two trials (REAL-LATE and ZEST-LATE trials were merged), a total of 2701 patients who had received DESs and had been free of major adverse cardiac or cerebrovascular events and major bleeding for a period of at least 12 mo were randomly assigned to receive clopidogrel plus aspirin or aspirin alone.

In this trial, more than half of the patients received a sirolimus-eluting stent (SES, Cypher, Cordis) and the other half received a paclitaxel-eluting stent (PES, Taxus, Boston Scientific) or a zotarolimus-eluting stent (ZES, Endeavor, Medtronic). Thus, the study population underwent PCI with predominantly first-generation DESs.

The median duration of follow-up was 19.2 mo. The cumulative incidence of primary outcomes (composite of myocardial infarction or death from cardiac causes) at 2 years was 1.8% with dual antiplatelet therapy compared with 1.2% with aspirin monotherapy (HR = 1.65; 95%CI: 0.80-3.36; *P* = 0.17). The individual risks of myocardial infarction, stroke, stent thrombosis, need for repeat revascularization, major bleeding and death from any cause did not differ between the two groups. However, in the dual therapy group, there was a non-significant increase in the composite risk of myocardial infarction, stroke or death from any cause (HR = 1.73, *P* = 0.051) and in the composite risk of myocardial infarction, stroke or death from cardiac causes (HR = 1.84, *P* = 0.06, Table 1). This trial concluded that the use of dual antiplatelets for longer than 12 mo following DES implantation was not more effective than aspirin monotherapy in reducing the rate of myocardial infarction or death from cardiac causes.

Recently, the DES-LATE trial reported that in the patients who were on 12 mo dual antiplatelet therapy

without complications, an additional 24 mo of dual antiplatelet therapy *vs* aspirin alone did not reduce the risk of major composite hard endpoints (cardiac deaths, myocardial infarction or stroke)^[8].

The EXCELLENT trial: (Dual antiplatelet 6 mo *vs* 12 mo). Some previous registry data suggested that dual antiplatelets for less than 12 mo after DES implantation does not increase major adverse cardiac events (MACE) and that there was no apparent clinical benefit from dual antiplatelets for longer than 6 mo^[9-11]. Data comparing a shorter duration of dual antiplatelets compared with 12 mo of dual antiplatelets are very limited. The EXCELLENT (Efficacy of Xience/Promus *vs* Cypher to Reduce Late Loss After Stenting) trial from South Korea compared 6 mo *vs* 12 mo dual antiplatelet therapy following DES implantation^[12].

Following DES implantation, 1443 patients were randomly assigned to receive 6 mo or 12 mo dual antiplatelets. The primary endpoint was a target vessel failure (composite of cardiac death, myocardial infarction or ischemia-driven target vessel revascularization) at 12 mo.

The rate of target vessel failure at 12 mo was 4.8% in the 6 mo dual antiplatelet group and 4.3% in the 12 mo group (the upper limit of 1-sided 95%CI: 2.4%; *P* = 0.001 for non-inferiority with a predefined non-inferiority margin of 4.0%). Although stent thrombosis tended to occur more frequently in the 6 mo dual antiplatelets group than 12 mo group (0.9% *vs* 0.1%, HR = 6.02; 95%CI: 0.72-49.96; *P* = 0.10), the risk of death or myocardial infarction did not differ in the two groups. In the pre-specified subgroup analysis, target vessel failure occurred more frequently in the 6 mo dual antiplatelet group (HR = 3.16; 95%CI: 1.42-7.03; *P* = 0.005) in diabetic patients (Table 2).

This study population predominantly received an everolimus-eluting stent (EES, Xience or Promus, 74.8%)

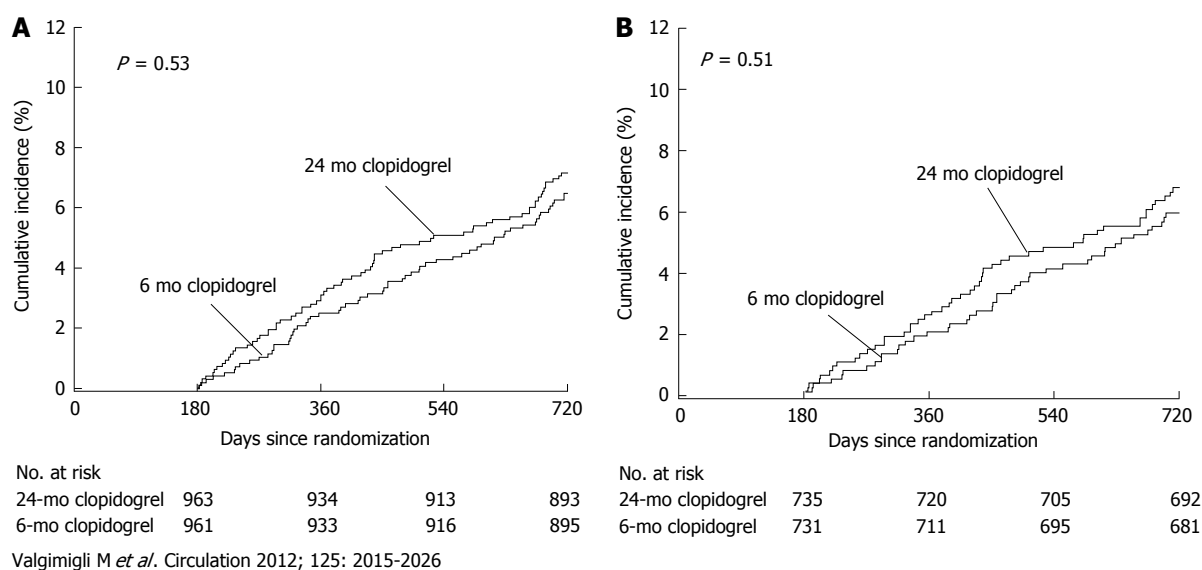


Figure 1 Landmark analyses of PRODIGY Trial^[13]. Cumulative rates of composite of death, myocardial infarction or cerebrovascular accident in all recruited patients (A) or in patients randomly allocated to the drug-eluting stent groups (B) using the 6 mo landmark analysis.

Table 2 Clinical outcomes of EXCELLENT trial n (%)

Clinical outcomes	6-mo DAPT ($n = 722$)	12-mo DAPT ($n = 721$)	HR ¹ (95%CI)	P
Target vessel failure ²	34 (4.8)	30 (4.3)	1.14 (0.70-1.86)	0.60
Total death	4 (0.6)	7 (1.0)	0.57 (0.17-1.95)	0.37
Cardiac death	2 (0.3)	3 (0.4)	0.67 (0.11-3.99)	0.66
Myocardial infarction	13 (1.8)	7 (1.0)	1.86 (0.74-4.67)	0.19
Death/myocardial infarction	17 (2.4)	14 (1.9)	1.21 (0.60-2.47)	0.58
Target vessel myocardial infarction	12 (1.7)	6 (0.8)	2.00 (0.75-5.34)	0.16
Cerebrovascular accident	3 (0.4)	5 (0.7)	0.60 (0.14-2.51)	0.48
Target lesion revascularization	17 (2.4)	18 (2.6)	0.94 (0.49-1.83)	0.86
Target vessel revascularization	22 (3.1)	22 (3.2)	1.00 (0.56-1.81)	0.99
Any revascularization	43 (6.2)	43 (6.2)	1.00 (0.66-1.53)	0.99
Stent thrombosis	6 (0.9)	1 (0.1)	6.02 (0.72-49.96)	0.10
Any bleeding	4 (0.6)	10 (1.4)	0.40 (0.13-1.27)	0.12
TIMI major bleeding	2 (0.3)	4 (0.6)	0.50 (0.09-2.73)	0.42
MACCE ³	56 (8.0)	60 (8.5)	0.94 (0.65-1.35)	0.72
Safety end point ⁴	24 (3.3)	21 (3.0)	1.15 (0.64-2.06)	0.64

The percentages shown are Kaplan-Meier estimates from the intention-to-treat analysis. ¹HRs are for the 6 mo *vs* 12 mo DAPT group; ²Target vessel failure was a composite of cardiac death, myocardial infarction or target vessel revascularization; ³MACCE was a composite of death, myocardial infarction, stroke or any revascularization; ⁴Safety end point was a composite of death, myocardial infarction, stroke, stent thrombosis or TIMI major bleeding. (Modified from Ref. [12]). DAPT: Dual antiplatelet therapy; TIMI: Thrombolysis in myocardial infarction; MACCE: Major cardiocerebral event.

and rest of the patients received SES (25.2%). The study population was heterogeneous in terms of different DESs, particularly first *vs* second generation DESs.

They concluded that 6 mo of dual antiplatelets did not increase the risk of target vessel failure at 12 mo after DES implantation compared with 12 mo of dual antiplatelets.

Although 6 mo of dual antiplatelets cannot be recommended in the general population on the basis of this trial, this may be helpful for physicians to decide the duration of dual antiplatelets case by case in clinical practice.

PRODIGY trial: (Dual antiplatelets 6 mo *vs* 24 mo). The purpose of the PRODIGY trial (Prolonging Dual Antiplatelets Treatment After Grading Stent-Induced Intimal Hyperplasia) was to assess the effect of dual antiplatelets for 6 mo *vs* 24 mo on long-term clinical outcomes after PCI in a broad all-comers patient population receiving a balanced DES or base-metal stent (BMS)^[13].

They randomly assigned 2013 patients to receive BMS, ZES, PES or EES. At 30 d, each stent group was randomly allocated to receive up to 6 mo or 24 mo of clopidogrel therapy in addition to aspirin.

The cumulative risk of the primary outcome (composite of death of any cause, myocardial infarction or cerebrovascular accident) at 2 years was 10.1% in the 24 mo dual antiplatelet group compared with 10.0% in the 6 mo group (HR = 0.98; 95%CI: 0.74-1.29; $P = 0.91$, Figure 1). The individual risks of death, myocardial infarction, cerebrovascular accident or stent thrombosis did not differ between the two groups; however, there was a consistently greater risk of hemorrhage in the 24 mo group. They concluded that a regimen of 24 mo of clopidogrel therapy in patients who had received a balanced mixture of DES or BMS was not significantly more effective than a 6 mo regimen in reducing the composite of death from any cause, myocardial infarction or cerebrovascular accident.

Table 3 Two year clinical outcomes of TWENTE trial *n* (%)

	Resolute ZES (<i>n</i> = 695)	Xience V EES (<i>n</i> = 692)	Difference (95%CI)	<i>P</i>
Target vessel failure	75 (10.8)	80 (11.6)	-0.8 (-4.1 to 2.6)	0.65
Death				
Any cause	29 (4.2)	33 (4.8)	-0.6 (-2.8 to 1.6)	0.59
Cardiac cause	11 (1.6)	19 (2.7)	-1.2 (-2.7 to 0.4)	0.14
Target vessel-related myocardial infarction				
Any	37 (5.3)	39 (5.6)	-0.3 (-2.7 to 2.1)	0.80
Q-wave	8 (1.2)	9 (1.3)	-0.2 (-1.3 to 1.0)	0.80
Non-Q-wave	29 (4.2)	30 (4.3)	-0.2 (-2.3 to 2.0)	0.88
Clinically indicated target vessel revascularization				
Any	39 (5.6)	35 (5.1)	0.6 (-1.8 to 2.9)	0.65
Target lesion failure	73 (10.5)	68 (9.8)	0.7 (-2.5 to 3.9)	0.68
Clinically indicated target lesion revascularization				
Any	34 (4.9)	18 (2.6)	2.3 (0.3 to 4.3)	0.03
Death from cardiac causes or target vessel myocardial infarction	46 (6.6)	53 (7.7)	-1.0 (-3.8 to 1.7)	0.45
Major adverse cardiac events ¹	90 (12.9)	82 (11.8)	1.1 (-2.4 to 4.6)	0.53
Patient-oriented composite endpoint ²	114 (16.4)	118 (17.1)	-0.7 (-4.6 to 3.3)	0.75
Stent thrombosis				
Definite (0-720 d)	6 (0.9)	1 (0.1)	0.7 (-0.0 to 1.5)	0.12
Definite or probable (0-720 d)	8 (1.2)	10 (1.4)	-0.3 (-1.5 to 0.9)	0.63
Definite, probable, or possible (0-720 d)	14 (2.0)	20 (2.9)	-0.9 (-2.5 to 0.8)	0.29
Very late definite or probable (361-720 d)	2 (0.3)	2 (0.3)	0 (-0.6 to 0.6)	1.00

Values are *n* (%). ¹Major adverse cardiac events is a composite of all-cause death, any myocardial infarction, emergent coronary artery bypass surgery and clinically indicated target lesion revascularization; ²Patient-oriented composite endpoint is a composite endpoint of all-cause death, any myocardial infarction and any revascularization. (Modified from Ref. [17]). ZES: Zotarolimus-eluting stent; EES: Everolimus-eluting stent.

TWENTE Trial: (Discontinuation of dual antiplatelets after 12 mo in ZES and EES). Second-generation DESs, such as EES (Xience V, Abbott Vascular, Santa Clara, California) and ZES (Resolute ZES, Medtronic Inc, Santa Rosa, California), were developed to improve clinical outcomes by overcoming the limitations of first generation DESs^[14,15]. The randomized TWENTE (The Real-World Endeavor Resolute *vs* Xience V DES Study in Twente) trial is an investigator-initiated study performed in a population with many complex patients and lesions and only limited exclusion criteria^[16]. Patients were randomly assigned 1:1 to ZES (*n* = 697) or EES (*n* = 694).

Two year follow up information was available on all patients. A strict policy of discontinuation of dual antiplatelets after 12 mo was followed, which is of interest for the present pre-specified 2 year analysis of clinical outcomes^[17]. The rate of continuation of dual antiplatelets beyond 12 mo was very low (5.4%). The primary

endpoint of target vessel failure, a composite of cardiac death, target vessel-related myocardial infarction and target vessel revascularization, did not differ between ZES and EES (10.8% *vs* 11.6%, *P* = 0.65), despite fewer TLRs in patients with EES (2.6% *vs* 4.9%, *P* = 0.03). The patient-oriented composite endpoint was similar (16.4% *vs* 17.1%, *P* = 0.75). Two year rates of definite or probable stent thrombosis were 1.2% and 1.4%, respectively (*P* = 0.63). Very late definite or probable stent thrombosis only occurred in 2 patients in each study arm (0.3% *vs* 0.3%, *P* = 1.00, Table 3).

They concluded that after 2 years of follow-up and stringent discontinuation of dual antiplatelets beyond 12 mo, Resolute ZES and Xience V EES showed similar results in terms of safety and efficacy for treating patients with a majority of complex lesions and off-label indications for DESs.

Other recent clinical reports

Kotani *et al*^[18] recently reported 5 year follow up results after SES implantation. They analyzed a prospective registry of 2050 patients with SES during a 5 year follow-up. A total of 1691 patients were divided into two groups: dual antiplatelets ≤ 12 mo, *n* = 749 and dual antiplatelets > 12 mo, *n* = 942 and compared the clinical outcomes using a landmark analysis. The frequencies of MACE (15.6% *vs* 18.2%), death (10.0% *vs* 11.5%), myocardial infarction (2.3% *vs* 2.1%), TLR (4.5% *vs* 11.5%) and stent thrombosis (0.8% *vs* 0.8%) were similar between the two groups. However, with regards to bleeding, an increase in the frequency of hemorrhage events was observed after 4 years from the index procedure in the dual antiplatelets > 12 mo group. They concluded that dual antiplatelets beyond 12 mo was associated with an increased frequency of bleeding complications and does not prevent the incidence of MACE, including stent thrombosis, during 5 years follow-up after SES implantation.

A recently published meta-analysis also supports a shorter duration of dual antiplatelets for both safety and efficacy following DES implantation^[19]. They searched for randomized controlled trials that compared longer *vs* shorter dual antiplatelet duration after DES implantation from the database inception to December 2011. Three randomized controlled trials comparing 5622 patients were included. Compared with short-term therapy, longer dual antiplatelet duration had a pooled OR of 1.26 (95%CI: 0.88-1.80; *P* = 0.21, random-effects) for the primary outcomes of cardiac death, myocardial infarction or stroke; OR = 1.29 (95%CI: 0.85-1.93; fixed-effects) for all-cause death; 1.23 (95%CI: 0.78-1.93; fixed-effects) for cardiac death; 0.91 (95%CI: 0.58-1.42; random-effects) for myocardial infarction; and 1.93 (95%CI: 1.01-3.69; fixed-effects) for stroke and 2.51 (95%CI: 1.10-5.71, fixed-effects) for thrombolysis in myocardial infarction major bleeding. The number needed to treat for an additional harmful outcome was 217.6 for stroke and 243 for thrombolysis in myocardial infarction major bleeding. This meta-analysis provides no evidence of benefits with longer dual antiplatelet duration compared with a shorter

course of therapy. It also reports significant harm with respect to major bleeding and stroke associated with prolonged dual antiplatelet use.

Another new clinical trial (OPTIDUAL; OPTImal DUAL antiplatelet therapy trial) is ongoing to assess the efficacy and safety of 12 vs 48 mo of dual antiplatelet therapy after DES implantation^[20].

Lastly, regarding clinical events associated with stent thrombosis, P2Y₁₂ and thromboxane receptor are not the sole therapeutic measure to prevent the thrombotic risk. There must be different pathways leading to thrombotic events, including hypersensitivity reactions^[21,22].

CONCLUSION

Despite the latest PCI guidelines recommending at least 1 year of dual antiplatelet therapy, recent randomized clinical trials, registries and meta-analysis data have shown that a shorter duration of dual antiplatelet therapy is as effective as a longer duration of dual antiplatelets, regardless of DES type (whether first-generation or next generation). Furthermore, a shorter duration of dual antiplatelets was associated with less bleeding complications without increasing the incidence of stent thrombosis. Currently, at least 6 mo of dual antiplatelets following next-generation DES implantation appears to be safe and effective, even with the expanded indication in the contemporary PCI setting. However, caution should be exercised until enough clinical data is obtained, in particular in the subset of higher risk patients, including diabetes, aspirin and clopidogrel resistance or the very complex lesion subset expecting a vulnerability to stent thrombosis. In this review, we focused only on classical dual antiplatelets, aspirin and clopidogrel. However, more data is needed to define the role of newer generation P2Y₁₂ inhibitors, including ticagrelor and prasugrel, especially in the acute coronary syndrome setting in the future.

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Arrhythmogenic ventricular cardiomyopathy: A paradigm shift from right to biventricular disease

Ardan M Saguner, Corinna Brunckhorst, Firat Duru

Ardan M Saguner, Corinna Brunckhorst, Firat Duru, Department of Cardiology, University Heart Center, CH-8091 Zurich, Switzerland

Author contributions: Saguner AM, Brunckhorst C and Duru F equally contributed to this article.

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Correspondence to: Firat Duru, Professor, Department of Cardiology, University Heart Center, Rämistrasse 100, CH-8091 Zurich, Switzerland. fiat.duru@usz.ch

Telephone: +41-44-2553565 Fax: +41-44-2554401

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Abstract

Arrhythmogenic ventricular cardiomyopathy (AVC) is generally referred to as arrhythmogenic right ventricular (RV) cardiomyopathy/dysplasia and constitutes an inherited cardiomyopathy. Affected patients may succumb to sudden cardiac death (SCD), ventricular tachyarrhythmias (VTA) and heart failure. Genetic studies have identified causative mutations in genes encoding proteins of the intercalated disk that lead to reduced myocardial electro-mechanical stability. The term arrhythmogenic RV cardiomyopathy is somewhat misleading as biventricular involvement or isolated left ventricular (LV) involvement may be present and thus a broader term such as AVC should be preferred. The diagnosis is established on a point score basis according to the revised 2010 task force criteria utilizing imaging modalities, demonstrating fibrous replacement through biopsy, electrocardiographic abnormalities, ventricular arrhythmias and a positive family history including identification of genetic mutations. Although several risk factors for SCD such as previous cardiac arrest, syncope, documented VTA, severe RV/LV dysfunction and young age at manifestation have been identified, risk stratification still needs improvement, especially in

asymptomatic family members. Particularly, the role of genetic testing and environmental factors has to be further elucidated. Therapeutic interventions include restriction from physical exercise, beta-blockers, sotalol, amiodarone, implantable cardioverter-defibrillators and catheter ablation. Life-long follow-up is warranted in symptomatic patients, but also asymptomatic carriers of pathogenic mutations.

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Key words: Arrhythmogenic right ventricular dysplasia/cardiomyopathy; Arrhythmias; Ventricular tachycardia; Sudden cardiac death; Implantable cardioverter defibrillator

Core tip: This manuscript constitutes an updated overview about arrhythmogenic ventricular cardiomyopathy (AVC) and describes well the paradigm shift in the understanding of AVC from an isolated right-sided entity to biventricular disease that can present with multiple facets. The most recent advances in molecular and clinical research are discussed, with particular focus on genetic novelties and risk stratification. We believe that this review will help clinicians to better understand the pathomechanisms that lead to AVC, its diagnosis and state-of-the-art therapeutic decision making.

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INTRODUCTION

Arrhythmogenic ventricular cardiomyopathy (AVC), as

recently re-named by the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA) consensus statement paper^[1], is generally referred to as arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D), constituting a hereditary cardiomyopathy usually with an autosomal-dominant inheritance pattern. Its first description by Giovanni Maria Lancisi, the Pope's physician, dates back to 1736 in his book "De Motu Cordis et Aneurysmatibus"^[2]. The first comprehensive description of ARVC/D by Guy Fontaine in 1978 marks a milestone for our current understanding of this heterogeneous disease^[3]. Initially, ARVC/D was thought to be an embryological aberration, such as Uhl's anomaly leading to the original designation of dysplasia^[4]. However, further research shed light on the pathophysiology of ongoing genetically determined myocardial atrophy that did not support the theory of a congenital myocardial absence. Thus, in 1995, ARVC/D was assigned to the World Health Organization's definition and classification of primary cardiomyopathies^[5]. Autopsy studies have been crucial in understanding AVC. Progressive atrophy of the ventricular musculature due to cumulative myocyte loss and infiltration by fibrous and adipose tissue can be observed.

The right ventricle (RV) is primarily affected in AVC, representing the most common form known as ARVC/D, and thus can be referred to as classic AVC^[6]. At a later stage, the left ventricle (LV) can also be involved and is often associated with severe disease and a worse prognosis^[7]. Advanced molecular genetic studies have identified causative mutations in genes encoding proteins of the intercalated disk, mainly desmosomal proteins^[8] that lead to reduced electrical and mechanical stability of the myocardium^[9,10]. Subsequent myocardial inflammation, apoptosis and necrosis may occur. Some of these histological changes are currently discussed as potential cases of myocarditis mimicking AVC^[11-14]. Because of the genetic basis and the many facets of the disease, the term "ARVC" is somewhat misleading. Particularly as biventricular involvement and less often isolated LV involvement may be present in a substantial proportion of patients^[15], a broader term such as "arrhythmogenic cardiomyopathy" should be preferred, as already suggested by Gallo *et al*^[16] almost 20 years ago, and as recently proposed by the HRS and the EHRA^[1]. However, the cardiology community is still reluctant to accept the proposed new nomenclature, probably because RV involvement constitutes a hallmark of the disease and non-classic forms are difficult to distinguish from non-ischemic dilated cardiomyopathies.

EPIDEMIOLOGY

In most parts of the world, phenotypic expression is more common in men than in women (2-3:1)^[17,18]. AVC commonly manifests during late childhood or adolescence but can also emerge in the elderly^[19,20]. With a general prevalence of 1:2000, which can be higher in certain geographical regions with enhanced genetic prevalence

such as the Veneto region or the Greek island Naxos, it is not so rare^[21,22]. Recent data indicates that the prevalence is even higher than initially estimated^[23]. AVC is recognized as a leading cause of sudden cardiac death (SCD) in young adults ≤ 35 years of age and may account for up to 10% of cardiovascular deaths in the < 65 age group^[24,25]. Of note, in one series from northern Italy, AVC accounted for up to 22% of SCD in all young adults ≤ 35 years of age^[26-29]. AVC usually first manifests with ventricular tachyarrhythmias (VTA) or SCD. In its most common form ARVC/D, ventricular arrhythmias originate in the RV and thus have left bundle branch block (LBBB) morphology^[28,30]. Less often, the primary manifestation can be heart failure without symptomatic arrhythmias. As LV function is often preserved at early stages, ventricular tachycardia (VT) may be asymptomatic as far as it does not degenerate into ventricular fibrillation (VF)^[29]. An early concealed phase without gross structural abnormalities is unique among the primary cardiomyopathies. On the contrary, in hypertrophic cardiomyopathy, arrhythmic risk can be ascribed to the underlying myocardial disarray. In dilated cardiomyopathy (DCM), arrhythmias generally concur with significant LV systolic dysfunction^[31]. Of note, early AVC may resemble myocardial channelopathies, such as Brugada syndrome (Bs)^[32], thus making correct diagnosis and risk stratification difficult.

DISEASE SUBTYPES

Classification of AVC into three different subtypes is evolving. AVC in its classic right-dominant form is the most common and best known and referred to as ARVC/D. The non-classic forms were first described by pathologists on autopsy studies and in isolated clinical case reports^[33,34]. Through intensive *in vivo* characterization of affected families, a link to hereditary mutations of the intercalated disk was established^[35-37]. LV involvement is increasingly described with a prevalence of up to 76% of cases, which may be attributed to improved diagnostic methods such as genetic testing, high-resolution contrast-enhanced cardiac magnetic resonance tomography (CMR), and recently the new technology of echocardiographic strain imaging^[38]. The proposed classification below is simplistic since due to genetic heterogeneity and epigenetic factors, a phenotypic continuum with right- and left-dominant subtypes at opposite ends has to be assumed.

In classic right-dominant ARVC, a dilated RV with fibro-fatty infiltration with no or only minimal LV involvement can be found at autopsy (Figure 1). This fibro-fatty infiltration typically begins subepicardially and may expand transmurally over time^[39]. Papillary muscles and trabeculae are generally not involved in this process^[25]. Yet, fatty infiltration alone does not constitute a pathognomonic sign of AVC, as a certain amount of epicardial and intramyocardial fat without an increase in fibrous tissue is present in both ventricles, more commonly in the RV, of persons without cardiovascular disease, particu-

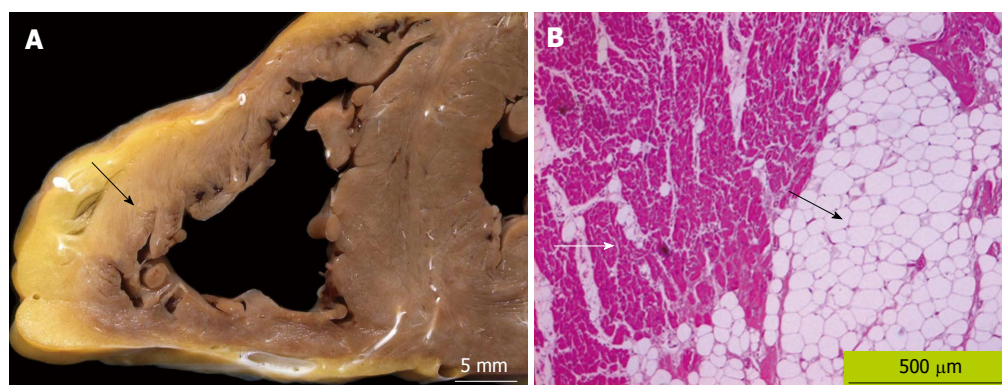


Figure 1 Typical pathology findings in arrhythmogenic ventricular cardiomyopathy/dysplasia. A: Macroscopic finding in a patient with arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D). The myocardium of the right ventricular free wall is partially replaced by fibro-fatty tissue (black arrow) that typically begins in the epicardial region and at later stages expands transmurally; B: Endomyocardial biopsy from a patient with ARVC/D demonstrating fatty (black arrow) replacement of the right ventricular myocardium. Strands of myocardium are still visible (white arrow, heidenhain trichrome, magnification $\times 60$).

larly in the obese and elderly^[17,40,41]. Another consistent finding in AVC is myocardial atrophy. Myocardial wall thinning, but also thickening, can both be seen on macroscopic examination^[22,40]. The subtricuspid region and the thin RV outflow tract (RVOT) are particularly prone to ventricular bulging and aneurysm formation that is present in 20%-50% of autopsy cases of ARVC/D^[39]. The former concept of early RV apical involvement and the term “triangle of dysplasia” have recently been questioned^[42]. Even although not very specific, ventricular aneurysms are strongly associated with the disease. The fact that the interventricular septum is rarely affected by fibro-fatty infiltration is an important disadvantage of endomyocardial biopsies, usually obtained from the septum, which may frequently yield false-negative results^[43]. If an affected region can be obtained for histological evaluation, it may reveal both replacement fibrosis, a repair mechanism after myocyte loss, and interstitial fibrosis, a reactive process, *e.g.*, to inflammation^[36,39].

Biventricular AVC is characterized by early and parallel involvement of both ventricles that can only be visualized by advanced imaging techniques such as contrast CMR or strain echocardiography^[36,44]. Progressive disease is characterized by systolic impairment and biventricular dilation with clinical features of global congestive heart failure. In contrast to other cardiomyopathies with biventricular involvement, ventricular arrhythmias of both right bundle branch block (RBBB) and LBBB configuration are present at an early stage, with around 10% of patients presenting with both^[31].

Left-dominant AVC (ALVC) has recently been suggested as a distinct form of AVC and is characterized by the early occurrence of LV involvement, while global RV function is preserved^[36]. An overlap with idiopathic myocardial fibrosis (IMF) accounting for certain SCD cases in a post mortem series has been reported^[45]. Typically, IMF features diffuse interstitial and replacement fibrosis with a predilection for the inferior LV wall in the absence of coronary artery disease and other structural abnormalities. Of note, myocardial infiltration by adipocytes is lacking in IMF. In biventricular disease or ALVC,

ventricular arrhythmias may also originate from the LV and thus show a RBBB configuration. Structural and electrocardiographic (ECG) findings are the left-sided analogues to those observed in ARVC/D (Table 1). The RV to LV ratio typically remains < 1.0 . To better understand ALVC and its clinical course, future investigations will be required.

PATHOGENESIS

Genetically-determined disruption of intercalated-disk integrity is a key factor promoting the development of AVC and SCD. This is widely named the “defective desmosome” hypothesis^[46,47]. Recent data indicates that loss of desmosomal integrity can substantially affect gap junctions, sodium channel function and electrical propagation at the micro- and nano-scale, thereby promoting ventricular arrhythmias in the absence of overt structural damage^[48]. Accordingly, lethal arrhythmias such as VF and polymorphic VT often occur during these concealed early stages, while sustained monomorphic VT occur at later stages, where there is enough substrate for macro-re-entry. Delmar *et al.*^[9] thus have postulated that mutations in desmosomal genes may affect the integrity of other molecular complexes that reside in proximity to desmosomes, such as connexins and voltage gated sodium channels, and are crucial for electrical synchrony. This molecular complex and its interactions have been named the cardiac connexome^[49,50]. Yet, genetic mutations in gap junctions such as connexin-43 have not been associated with AVC so far^[10,51].

Currently, two theories for the understanding of progressive fibro-fatty replacement of the myocardium exist: (1) inflammation as a response to myocardial injury^[4,25,39]. Lymphocytic interstitial infiltrates surrounding foci of necrotic or degenerative myocytes are observed on histopathology. Myocyte cell death may occur *via* apoptosis or necrosis underlying chronic inflammation. Acute myocyte cell death has also been reported, suggesting acute myocarditis during the disease course^[52]. Periodic exacerbations of a previously quiescent disease may be

Table 1 Characteristics of arrhythmogenic ventricular cardiomyopathy

	Classic right dominant form (ARVC/D)	Left dominant form
12-lead surface ECG	Intraventricular conduction delay in V1-V3 QRS complex prolongation V1-V3 ϵ wave in V1-V3 (Incomplete) RBBB Inverted T-waves in V1-V3 Inverted T-waves in V1-V6 with biventricular involvement ST elevation in V1-V3 Poor R wave progression	Leftward QRS axis ($< 0^\circ$) ϵ like waves in inferior or lateral leads LBBB Inverted T-waves in infero-lateral leads Inverted T-waves V1-6 with biventricular involvement - -
Signal-averaged ECG	Late potentials	-
Arrhythmia	PVC/VT of LBBB configuration	PVC/VT of RBBB configuration
Ventricular volumes	Mild to severe RV-dilation \pm dysfunction	Mild to severe LV-dilation \pm dysfunction
RV/LV volume ratio	≥ 1.2 , increases with disease expression	< 1.0
Other imaging abnormalities	Regional wall motion abnormalities in RV RV aneurysms Fat/LGE in RV myocardium	Regional wall motion abnormalities in LV Non-compacted appearance LGE in the subepicardial and midwall LV myocardium
Genetics	Affected genes currently known to be associated with AVC	Association with TMEM43 and phospholamban mutations ^[1]

Adapted from Jacoby *et al*^[64]. ARVC/D: Arrhythmogenic right ventricular cardiomyopathy/dysplasia; ECG: Electrocardiogram; ϵ : Epsilon; LBBB: Left bundle branch block; LGE: Late gadolinium enhancement; LV: Left ventricle; PVC: Premature ventricular contraction; RBBB: Right bundle branch block; RV: Right ventricle; VT: Ventricular tachycardia; AVC: Arrhythmogenic ventricular cardiomyopathy; TMEM43: Transmembrane protein 43.

triggered by such inflammatory episodes and are called “hot phases” of AVC. Occasionally, these phases may clinically present with chest pain, dynamic ECG changes and increased arrhythmic activity^[51]. Strenuous physical activity can trigger inflammation as mechanical stress to the impaired intercalated disk leads to myocyte detachment and myocyte cell death^[53]. It is important to keep in mind that isolated myocarditis, sarcoidosis, Bs and other diseases can mimic AVC^[14], which may prompt further histological and molecular investigations. If molecular genetic analyses or pedigree analyses of affected family members are not performed, a biopsy specimen may be classified as focal myocarditis^[56]. Yet, previous studies have indicated a link between AVC and a susceptibility to viral and bacterial myocarditis, particularly in non-hereditary forms^[54,55]. The prevalence of viral genome in myocardial biopsies from AVC patients is reported with a broad range from 0% to 75%, but a causal association is difficult to prove. Presence of enteroviral RNA has been reported in tissue from patients with DCM, suggesting an innocent bystander role. Nevertheless, viral presence may play a secondary yet important role in disease progression^[47], and (2) apoptosis following disruption of the intercalated disc^[56] with electromechanical instability, as indicated by detection of fragmented DNA, expression of protease CPP-32 by immunohistochemistry and positive Tc-annexin V scintigraphy *in vivo*^[11-14,57,58]. These histological disarrangements create a substrate for electrical re-entrant phenomena and delayed ventricular activation triggering ventricular arrhythmias. Of note, as AVC can cause ventricular arrhythmias and SCD in the absence of gross macroscopic abnormalities, histological and molecular examinations are important to establish a post-mortem diagnosis^[59]. Other investigators observed that epicardium-derived cell cultures obtained from neonatal hearts lacking plakophilin-2 (PKP2), an important desmosomal gene, revealed enhanced cell migration velocity

and proliferation, leading to the hypothesis that desmosomal mutations may cause infiltration of fibroblasts and adipocytes from the epicardial cell layer into the myocardium^[60]. This hypothesis is consistent with the frequent clinical observation that fibro-fatty infiltration progresses from the epicardium towards the endocardium.

GENETICS

Analyses of the first- and second-degree relatives of patients suggest that up to 50% of AVC cases are familial^[61,62]. AVC is most commonly inherited as a Mendelian autosomal dominant trait with incomplete penetrance^[46,47], although two autosomal recessive forms have been described^[63-65]. To date, 12 different AVC loci are reported in the Online Mendelian Inheritance in Man (Table 2)^[66]. Compound and digenic heterozygosity has been recently suggested, indicating that in some cases more than one pathogenic allele may be involved in the disease process^[65,67,68]. As penetrance is incomplete, genetically affected relatives often demonstrate variable and mild phenotype and the prevalence of familial disease is often underestimated in clinical practice^[31,62]. The fact that AVC can be inherited has been known since 1982 after the description of 24 adult cases, two in the same family, by Marcus *et al*^[69]. Six years later, the autosomal dominant pattern of inheritance with incomplete penetrance and variable expression was demonstrated in a study of nine Italian families^[26]. As patients with fully penetrant cardiomyopathy and readily discernible features of the palms, plantar fascia and hair were clustered in families on the Greek island Naxos, an autosomal recessive mutation in the desmosomal protein junction plakoglobin (JUP) was finally discovered, which became known as Naxos disease. Myocytes and epidermal cells share similar intercalated disks (desmosomes and fascia adherens) and are both exposed to high shear stress, the

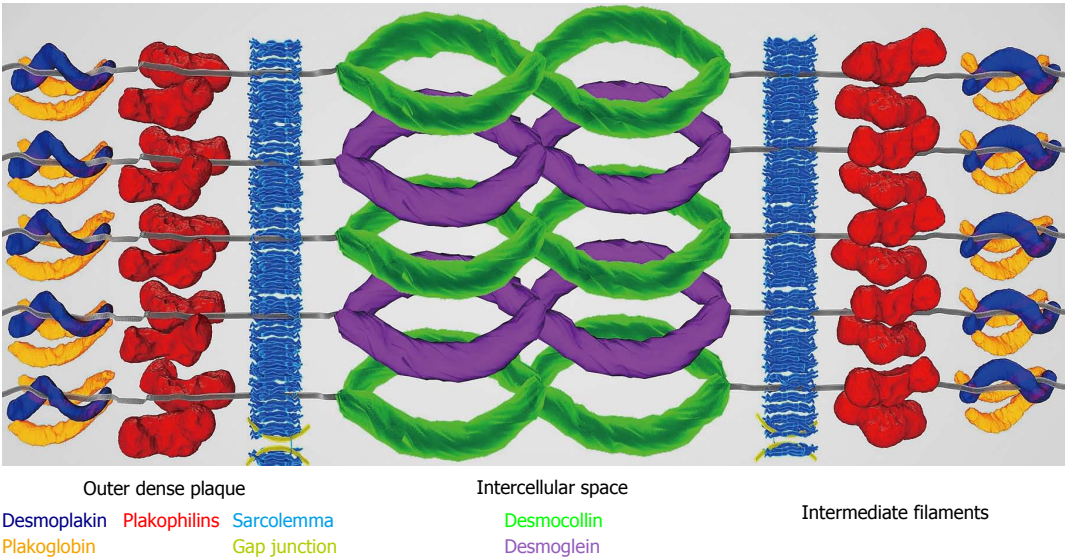


Figure 2 Molecular model of the desmosome: in the desmosomal complex the intermediate filaments of the cytoskeleton (desmin in the heart) are linked to the transmembranous cadherins (desmocollin and desmoglein) *via* armadillo proteins (plakoglobin and plakophilin) and desmoplakin. This interaction is crucial for myocardial mechanical and electrical stability. Mutations in arrhythmogenic right ventricular cardiomyopathy mostly affect desmosomal proteins.

Table 2 Arrhythmogenic ventricular cardiomyopathy classification, from OMIMTM Online Mendelian inheritance in Man			
AVC subtype	Chromosome/locus	Mode of transmission	Encoded protein
ARVC/D 1	14q23-q24	Autosomal-dominant	TGFβ3
ARVC/D 2	1q42-q43	Autosomal-dominant	RyR2
ARVC/D 3	14q12-q22	Autosomal-dominant	-
ARVC/D 4	2q32	Autosomal-dominant	TTN
ARVC/D 5	3p23	Autosomal-dominant	TMEM43
ARVC/D 6	10p12-p14	Autosomal-dominant	-
ARVC/D 7	10q22	Autosomal-dominant	-
ARVC/D 8	6p24	Autosomal-dominant	DSP
ARVC/D 9	12p11	Autosomal-dominant	PKP2
ARVC/D 10	18q12	Autosomal-dominant	DSG2
ARVC/D 11	18q12.1	Autosomal-dominant	DSC2
ARVC/D 12	17q21	Autosomal-dominant	JUP
Naxos disease	17q21	Autosomal-recessive	JUP

AVC: Arrhythmogenic ventricular cardiomyopathy; ARVC/D: Arrhythmogenic right ventricular cardiomyopathy/dysplasia; TGF: Transforming growth factor; RyR2: Ryanodine receptor 2; TTN: Titin; TMEM43: Transmembrane protein 43; DSP: Desmoplakin; PKP2: Plakophilin-2; DSG2: Desmoglein-2; DSC2: Desmocollin-2; JUP: Junction plakoglobin.

heart particularly during strenuous physical activity and increased cardiac workload. Thus, it has been assumed that common genes encoding proteins of the intercalated disk might be responsible for AVC. In 1994, the first chromosomal locus (14q23-q24) for autosomal dominant AVC was reported in Italy^[47]. Linkage analyses shed light on its genetic heterogeneity with sequential discovery of several loci on chromosomes 1, 2, 3, 6, 10, 12, 14, 17 and 18 (Table 2). Most frequently, mutations in genes encoding components of the cardiac desmosome, an important protein complex of the intercalated disk (Figure 2), are associated with AVC, resulting in impaired intercalated-disk integrity^[62,67,68]. The pathogenic importance of desmosomal mutations was confirmed by electron mi-

croscopy and immunohistochemistry^[2,56]. Intercellular junctions consist of a core region that mediates cell-cell adhesion and a plaque region that provides attachment to the intermediate filaments within the myocyte. Three groups of desmosomal proteins are known: (1) transmembrane desmosomal cadherins including desmocollins 2 and desmogleins 2 (DSG2); (2) desmoplakin (DSP), a plakin family protein that attaches directly to intermediate filaments (desmin in the myocardium); and (3) linker proteins such as armadillo family proteins including JUP (catenin-γ) and PKP2 that mediate interactions between the desmosomal cadherin tails and DSP^[70]. In about 80% of cases with confirmed pathogenic mutations, PKP2, DSP and DSG2 are altered^[22]. Besides desmosomal gene mutations, mutations in genes encoding proteins that interact with desmosomal proteins were found as well. These include: (1) the transforming growth factor β3 that conveys cytokine-stimulating fibrosis and modulates cell adhesion and growth^[52]; (2) the human ryanodine receptor 2 (RyR2) that induces the release of calcium from the myocardial sarcoplasmic reticulum and that is also associated with catecholaminergic polymorphic VT (CPVT)^[71]; (3) the transmembrane protein 43 (TMEM43) discovered in the Canadian Newfoundland founder population and Europe^[72] that functions as a PPAR-γ response element, an adipogenic transcription factor; (4) the intermediate filament desmin; (5) the tumor protein 63; and (6) recently, titin (TTN) that bridges the sarcomere along its longitudinal axis and forms a continuous filament along the myofibril^[66]. As TTN binds to the transitional junction of the intercalated disk, this may explain a functional link to the desmosome^[66,73,74]. Current molecular studies are screening other components of the desmosome and related proteins, such as plectin and pinin (Table 3)^[75]. NFκB interacting protein-1 is another extra-desmosomal gene of interest, which has been isolated in Poll-Here-

Table 3 Future candidate proteins for arrhythmogenic ventricular cardiomyopathy

Encoded protein
Components of the desmosome
Plectin
Emerin
Components of the adherens junction
β -catenin
α -catenin
N-cadherin
Components of the gap junction
Connexin 43
Myotonic dystrophy protein kinase-1
Laminin receptor-1
Components of dystrophin-glycoprotein complex

ford cattle with recessive AVC and woolly hair coat syndrome^[76]. Yet, the pathogenic role of NF κ B interacting protein-1 mutations in humans has to be demonstrated in future studies.

MODIFIER GENES AND ENVIRONMENTAL FACTORS

Although a plethora of pathogenic mutations exists, these mutations cannot account for the entire broad spectrum of disease expression. Data from the Newfoundland founder population and populations from the Dutch and Swiss ARVC/D registries show a strong male predominance of disease expression^[77]. A modifier effect of testosterone has been discussed. Yet, this male predominance has not been confirmed in the Johns Hopkins ARVC/D cohort, which may be associated with similar exercise levels among males and females in the United States. Nevertheless, outcomes were strongly gender dependent in all of those cohorts, with male gender constituting an independent risk factor for adverse outcomes^[37,62,72,78,79]. In one study, 67% of family members showed discordant disease patterns between RV and LV involvement^[31]. Recent data pointing at the importance of compound and digenic heterozygosity indicates that modifier genes may account for residual variation and disease severity^[68,80]. The first evidence for environmental influences in AVC arose from monozygotic twin studies, where differences were reported in symptom onset, structural severity and arrhythmic risk. Strenuous physical activity seemed to play an important role in these four cases^[81]. These preliminary observations were confirmed in two recent studies, in which endurance training and frequent exercise were associated with earlier disease manifestation and disease severity^[31,82]. Future studies will be crucial to distinguish between pathogenic mutations and innocent bystander mutations and to define the role of epigenetic factors in disease manifestation and progression. As recently proposed by the HRS/EHRA consensus statement, genetic testing should only be performed if the signal-to-noise ratio is expected to be $> 10^{[1]}$.

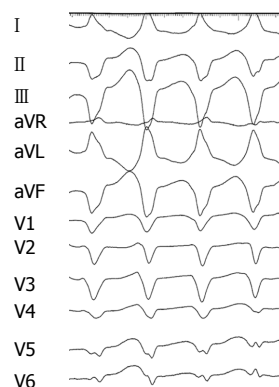


Figure 3 Monomorphic sustained ventricular tachycardia with left bundle branch block morphology and superior axis (II, III, aVF negative), a major criterion for arrhythmogenic right ventricular cardiomyopathy/dysplasia according to the revised 2010 task force criteria.

CLINICAL PRESENTATION

AVC has a reported community-based prevalence of 1 in 2000 and thus cannot be classified as a “rare” disease according to the 2007 European definition. These numbers reflect the importance of appropriate diagnostic tools as it is often underdiagnosed, particularly in early and mild cases. The above mentioned non-classic subtypes are usually not considered or misattributed as DCM. Some forms mimic myocarditis. Early disease with arrhythmias but without overt structural changes may be misjudged as idiopathic VT or ventricular ectopy^[36,46]. In the elderly, AVC is rarely considered as a differential diagnosis, which is certainly a false assumption. All these aspects infer that real-world prevalence is higher. In the following section, we provide an overview of clinical symptoms and signs that shall increase awareness of the disease, particularly in non-classic forms, for timely diagnosis and prevention of SCD. AVC should be suspected if the following symptoms or signs occur: (1) palpitations; (2) presumably arrhythmic presyncope or syncope; (3) VT with LBBB morphology; (4) aborted SCD. Palpitations and (pre)syncope are the most frequent symptoms^[17]. A high clinical suspicion should be raised if these symptoms correlate with premature ventricular contractions (PVC) or VT with LBBB morphology, particularly with a superior axis (Figure 3). However, ALVC or biventricular disease can present with VT with RBBB morphology or both (Table 1, Figure 3). The presence of monomorphic VT is associated with late disease stages, although gross structural changes are not mandatory^[28,83]. Recently, disease severity, VT frequency and early onset of VT have been associated with the presence of common desmosomal mutations, particularly if more than one pathogenic variant was present^[62,67,84]. Up to 25% of patients present with supraventricular tachycardia (SVT), most frequently atrial fibrillation, which is associated with male gender, increasing age and left atrial enlargement in AVC^[85]. SVT are very important as they are associated with inappropriate implantable cardioverter defibrillator (ICD) shocks

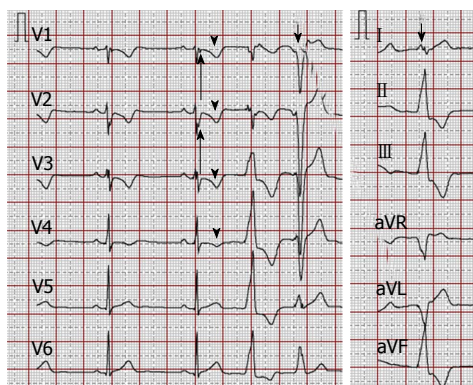


Figure 4 Electrocardiographic findings. A 12-lead surface electrocardiogram (25 mm/s, 10 mm/mV) showing typical depolarization abnormalities (prolonged terminal activation duration in V1-V2, a minor criterion according to 2010 task force criteria, long arrows) and repolarization abnormalities (T-wave inversions V1-V4 in the absence of complete right bundle branch block, a major criterion according to 2010 task force criteria, arrowheads), and premature ventricular contractions with two different morphologies (short arrows).

and an increased risk of both heart failure and death. Furthermore, atrial arrhythmias present at a younger age than in the general population^[86]. It is not rare that AVC first manifests as SCD, with some authors reporting an annual incidence of 9%^[87]. Whereas some authors report that SCD occurs preferentially during strenuous physical activity^[25,87,88], according to others it may often occur in the sedentary state^[13,25]. In ARVC/D caused by TMEM43 mutations, enhanced sympathetic activity as a trigger for lethal arrhythmias is established^[71]; (5) chest pain with or without dynamic ST elevation/T-wave changes on 12-lead surface ECG \pm rise in cardiac biomarkers; and (6) presumed DCM with early onset and frequent ventricular arrhythmias. Precordial T-wave inversions beyond V1 after puberty (Table 1, Figure 4) and T-wave inversions in the right precordial leads V1-V3 may potentially be benign, particularly before puberty. Their prevalence among athletes and sedentary controls is similar^[89], suggesting that this is not a training-related phenomenon. According to recent recommendations, a further evaluation with transthoracic echocardiography (TTE) may be performed after puberty. If imaging is inconclusive, regular follow-up by serial clinical examinations, ECG and TTE can be performed as structural alteration may become apparent after several years^[90,91]. RV failure with dyspnea and signs of right sided heart failure are rather rare and reported in up to 6% of patients at initial presentation. If the LV is involved, congestive heart failure may occur. Importantly, the clinician should be aware that AVC cannot be excluded by the absence of structural abnormalities as arrhythmias often occur in the “concealed phase” and structural abnormalities may follow after years. In a review reporting 37 families with AVC index patients, only 151 of 365 family members had clinically manifested disease and 17 family members were healthy despite a pathogenic mutation^[28]. Thus, genetic screening of family members may help to identify AVC, although a negative test does not exclude it.

DIAGNOSIS

Revised 2010 task force criteria

Currently, no gold standard to establish or exclude the diagnosis of AVC exists. In 2010, the original 1994 task force criteria (TFC) for diagnosis of ARVC/D by Marcus *et al.*^[92] were revised in order to enhance diagnostic sensitivity and particularly to improve identification of affected asymptomatic family members^[93]. The importance of pathogenic mutations was acknowledged and precise cut-off values for imaging and histological evaluation were provided. The impact of these changes is currently being evaluated. Some investigators report an increased diagnostic yield with the revised TFC^[94,95], while others could not demonstrate a benefit^[96,97]. It is important to keep in mind that these TFC only apply to ARVC/D with or without LV involvement. The revised TFC assign the findings into six categories (Table 4): (1) global and/or regional myocardial dysfunction and structural abnormalities; (2) histological characterization; (3) repolarization abnormalities on 12-lead surface ECG; (4) depolarization abnormalities on 12-lead surface ECG; (5) arrhythmias; and (6) family history and genetics.

Definite diagnosis requires 2 major criteria, 1 major and 2 minor criteria, or 4 minor criteria from different categories. ARVC/D is considered “borderline” if 1 major and 1 minor criterion, or 3 minor criteria are present. ARVC/D is still “possible” if 1 major criterion or 2 minor criteria are present. For each individual, comprehensive non-invasive evaluation is necessary. This includes a thorough clinical history and examination, pedigree analysis, 12-lead surface ECG, TTE with detailed assessment of the RV, CMR, stress testing in order to induce arrhythmias, and Holter ECG monitoring. If suspicion remains high and symptoms are rare, event recorders and invasive procedures may be needed.

Physical examination

Fifty percent of patients will have a normal physical exam. The other 50% will show abnormalities such as giant a-waves on the jugular veins, tricuspid regurgitation murmur, a fixed splitting of S2, and right-sided S3-S4 at the left sternal border with augmentation during inspiration in case of RV dilation^[88,98].

12-lead surface ECG and signal-averaged ECG

An abnormal 12-lead surface ECG will be present in about 50% of patients with ARVC/D. In one study, ECG was abnormal in 90% of patients after a follow-up period of 6 years^[99]. Abnormalities include epsilon waves, a QRS duration ≥ 110 ms in V1-V3, and T-wave inversions in the right precordial leads (Figure 4). A prolonged terminal activation duration (measured from the nadir of the S wave until the end of the QRS complex) in V1-V3 ≥ 55 ms is considered as a minor criterion for ARVC/D and has been reported as the first sign in young asymptomatic family members^[45,62,100]. However, interpretation of ECG findings, apart from T-wave inversions, significantly var-

Table 4 Revised (2010) task force criteria for diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia, adapted from Marcus *et al.*^[92]

	Structural alterations
Major	TTE regional RV akinesia, dyskinesia, or aneurysm and 1 of the following criteria (end diastole) PLAX RVOT ≥ 32 mm [(PLAX/BSA) ≥ 19 mm/m ²] PSAX RVOT ≥ 36 mm [(PSAX/BSA) ≥ 21 mm/m ²] Or RV fractional area change $\leq 33\%$ CMR regional RV akinesia, dyskinesia, or dyssynchronous RV contraction and 1 of the following criteria (end diastole) RV end-diastolic volume/BSA ≥ 110 mL/m ² (δ) or ≥ 100 mL/m ² (\varnothing) Or RV ejection fraction $\leq 40\%$ RV angiography regional RV akinesia, dyskinesia, or aneurysm
Minor	TTE regional RV akinesia, or dyskinesia and 1 of the following criteria (end diastole) PLAX RVOT ≥ 29 -31 mm [(PLAX/BSA) ≥ 16 -18 mm/m ²] PSAX RVOT ≥ 32 -35 mm [(PSAX/BSA) ≥ 18 -20 mm/m ²] RV fractional area change $> 33\%$ -39% CMR regional RV akinesia, dyskinesia, or dyssynchronous RV contraction and 1 of the following criteria (end diastole) RV end-diastolic volume/BSA ≥ 100 -109 mL/m ² (δ) or ≥ 90 -99 mL/m ² (\varnothing) Or RV ejection fraction $> 40\%$ -44%
Major	Histopathology (endomyocardial biopsy) Residual myocytes $< 60\%$ by morphometric analysis with fibrous replacement of the RV free wall myocardium ≥ 1 sample, with or without fatty replacement
Minor	Residual myocytes 60%-75% by morphometric analysis with fibrous Replacement of the RV free wall ≥ 1 sample
Major	Repolarization abnormalities (> 14 years of age) T-wave inversions V1-V3 or beyond (in absence of complete RBBB)
Minor	T-wave inversions V1-V2 or V4-V6 (in absence of complete RBBB) T-wave inversions V1-V4, if complete RBBB present
Major	Depolarization abnormalities Epsilon wave (reproducible low-amplitude signals between end of QRS complex to onset of the T-wave) in V1 to V3
Minor	SAECG with late potentials (if QRS complex on standard surface ECG < 110 ms) or terminal activation duration of QRS ≥ 55 ms in V1, V2 or V3
Major	Arrhythmias VT of LBBB morphology with superior axis
Minor	VT of RVOT configuration, LBBB morphology with inferior axis or of unknown axis > 500 PVC per 24 h (holter)
Major	Family history ARVC/D in a first-degree relative who meets current TFC ARVC/D confirmed pathologically at autopsy or surgery in a first-degree relative Identification of a pathogenic mutation categorized associated with ARVC/D in an index patient
Minor	Suspected ARVC/D in a first-degree relative-premature SCD (< 35 years of age) due to suspected ARVC/D in a first-degree relative ARVC/D confirmed pathologically or by current TFC in second-degree relatives

Definite diagnosis: two major or one major and two minor criteria or four minor from different categories; Borderline diagnosis: one major and one minor or three minor criteria from different categories; Possible diagnosis: one major or two minor criteria from different categories. BSA: Body surface area; CMR: Cardiac magnetic resonance tomography; LV: Left ventricle; PLAX: Parasternal long-axis view; PSAX: Parasternal short-axis view; RBBB: Right bundle branch block; RVOT: Right ventricular outflow tract; RV: Right ventricle, TTE: Transthoracic echocardiogram, PVC: Premature ventricular contraction VT: Ventricular tachycardia; SAECG: Signal-averaged electrocardiographic; LBBB: Left bundle branch block; ARVC/D: Arrhythmogenic right ventricular cardiomyopathy/dysplasia; TFC: Task force criteria; SCD: Sudden cardiac death.

ies among observers (unpublished data as yet from our group). This is particularly true for what is considered an epsilon wave. A limitation of T-wave inversions is the fact that they can also be found in healthy individuals, patients with anterior ischemia or RV hypertrophy^[90,101]. A recent study highlighted the importance of serial ECG evaluations as dynamic ECG changes occurred in 23% of patients over a median follow-up period of 34 mo, but these were not paralleled by structural abnormalities^[102]. Fibro-fatty infiltrations disrupt the electrical continuity of myocardial fibers. This leads to fragmentation and delay of ventricular depolarization (zig-zag pathways). On the surface, this may be visible as QRS fragmentation^[103], late ventricular potentials of small amplitude such as epsilon waves^[104], or late potentials recorded by signal-averaged ECG (SAECG)^[87,105]. An abnormal SAECG (a minor criterion) indicates progressive disease and may predict VT,

although a recent study has questioned the latter^[28,106]. SAECG may not be sensitive enough to detect early forms of AVC^[28].

Stress testing

Exercise can induce ventricular arrhythmias and is important in patients with suspected AVC. However, VT with LBBB morphology and inferior axis can occur in both ARVC/D and idiopathic RVOT-VT without underlying structural abnormalities^[107]. A recent study has proposed ECG criteria and a scoring system to distinguish between the two entities^[108].

Transthoracic echocardiography

In many centers, TTE constitutes the initial imaging tool for evaluation of patients with suspected AVC and for screening family members as it is readily available and

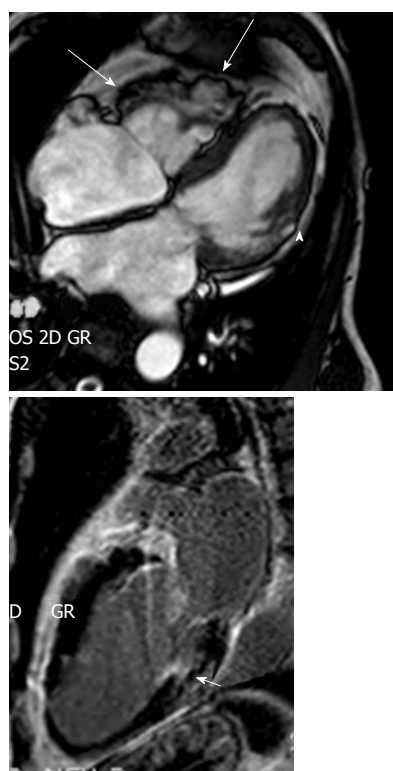


Figure 5 Regional right ventricular dyskinesia of the right free wall detected by cardiac imaging are considered as a major criterion for right ventricular cardiomyopathy/dysplasia according to the revised task force criteria if additionally right ventricle dilation or impaired right ventricle ejection fraction are present. These cardiac magnetic resonance images (upper panel 4-chamber view, lower panel 2-chamber view late sequences) show aneurysms of the RV free wall (long arrows), and LV involvement detected by a small akinetic region (arrowhead) and late gadolinium enhancement of the posterior LV wall (short arrow), confirming biventricular involvement. ARVC/D: arrhythmogenic right ventricular cardiomyopathy/dysplasia; RV: Right ventricle; LV: Left ventricle.

rapidly informative. It may demonstrate RV enlargement or multiple areas of dilation and regional contraction abnormalities, mainly in the subtricuspid region, RVOT and RV apex^[109]. According to the revised TFC, evaluation and measurements of the RVOT are crucial for diagnosis^[110]. The LV can also be affected, particularly in non-classic forms displaying hypokinesia and a reduced ejection fraction, although in most cases LV structural abnormalities are localized in the posterolateral region^[111,112].

CMR

CMR has emerged as the non-invasive diagnostic tool of choice for assessing the RV over the past 15 years^[45,113]. Besides highly accurate assessment of right sided volumes, myocardial mass and systolic and diastolic function, the contrast-enhanced CMR can reveal intramyocardial fibrosis by late gadolinium enhancement (LGE)^[114]. Yet, intramyocardial fat and fibrosis as diagnostic targets in AVC were not integrated in the revised TFC because of the limited specificity of these findings, particularly in the absence of regional wall motion abnormalities, significant intra- and inter-observer variability, and the need for highly specialized interpreters in visualizing the RV myocardium^[45,115,116]. In fact, it can be challenging to be certain

of LGE within the RV myocardium because of the thin RV and possible confusion with fat. The main difference in CMR criteria compared to the 1994 criteria constitutes the quantification of RV dilation and RV function. CMR plays an important role in diagnosing AVC (Figure 5) but consensus guidelines for non-classic forms are eagerly awaited. Some authors emphasize the importance of combining TTE with CMR to increase diagnostic yield. New diagnostic tools for detection of early diastolic and systolic abnormalities such as three-dimensional echocardiography, strain echocardiography and CMR tagging could facilitate early diagnosis of ACV^[117-120]. The promising results of these preliminary studies^[121,122] will have to be validated in large prospective studies.

RV angiography

RV angiography is considered a very useful test to diagnose classic forms of AVC and to evaluate RV function^[123,124]. Its positive predictive value is above 85%, with a negative predictive value of 95%^[88]. Technical aspects of the procedure can be found at *arvd.org*. Good quality images allow global and regional analyses of morphology and wall motion. RV angiography also has certain limitations that explain why it is not widely used in clinical practice. Clinicians want to offer non-invasive strategies without ionising radiation, particularly if patients are young. Additionally, serial follow-up RV angiographies for monitoring disease progression are difficult to perform. It is important to remember that according to the revised 2010 TFC, with all three imaging techniques, hypokinesia is no longer considered diagnostic.

Electrophysiological study and electroanatomical voltage mapping

Arrhythmias can be induced during an electrophysiological study (EPS) with programmed ventricular stimulation. Induction of clinical VT can guide ablation. The susceptibility for arrhythmias, arrhythmia detection, ICD treatment algorithms and efficacy of antiarrhythmic drugs can be assessed. Electroanatomical voltage mapping (EAM) is a technique using electrophysiological catheters to measure local myocardial voltages. After obtaining several hundred points, a voltage map can be reconstructed. According to several studies, healthy RV myocardium displays bipolar voltages > 1.5 mV^[125-127]. In myocardium infiltrated by fibro-fatty tissue, abnormally low voltages with a longer duration, splitting and fractionation of signals can be found. Myocardial voltage maps are usually obtained from the endocardium but epicardial measurements after puncturing the pericardial sac are also feasible. EAM has been shown to be safe and to improve outcomes of VT ablation in ARVC/D^[128-131]. The diagnostic and prognostic utility of EAM has not yet been implemented in the current TFC. Larger prospective studies may consolidate the role of EAM in the diagnostic armamentarium^[125,132,133].

Endomyocardial biopsy

Endomyocardial biopsy (EMB) was considered the di-

agnostic gold standard for AVC for a long time. It may allow confirmation of AVC in an index patient and exclude potential differential diagnoses such as sarcoidosis or Chagas disease. However, EMB are commonly taken from the thicker RV septum to assure a safe procedure. It was recognized that the septum is often spared by fibro-fatty infiltration and thus often yields false-negative results^[132,134]. Nevertheless, septal EMB can identify other conditions such as sarcoidosis, myocarditis and IMF. EMB from diseased regions is problematic as these regions are often difficult to reach, very thin and sample acquisition carries an increased risk of perforation and tamponade^[4]. Histological analysis should best be performed by an expert cardiac pathologist who judges the amount of surviving myocytes and fibro-fatty replacement. The results can be allocated as one major or one minor criterion according to the revised TFC. As AVC is patchy, several biopsies should be obtained. EAM-guided biopsies taken from low-voltage areas may improve diagnostic yield and better distinguish between myocarditis or sarcoidosis^[14,125,135]. Serious concerns remain about the hazards of sampling thin areas, although complication rates in preliminary studies were low^[14]. Moreover, EAM-guided EMB may be of limited value in early stages of AVC when serious arrhythmias occur in the absence of gross structural abnormalities. Additional immunohistochemical staining of the intercalated disk, *e.g.*, with plakoglobin, may turn into a valuable tool for pathologists in the future but results very much depend on the protocols used^[2]. Confirmation of typical histological changes by cardiac surgery or necropsy can help to confirm the diagnosis and exclude differential diagnoses.

Genetic testing

A consensus statement from the HRS and the EHRA regarding genetic testing in AVC was published recently^[1]. The major purposes of genetic testing are to confirm AVC in probands with a high (Class II a recommendation, level of evidence C) or intermediate (at least 1 major or 2 minor criteria; Class II b recommendation, level of evidence C) clinical suspicion and to identify genetically-affected relatives harboring the pathogenic mutation (Class I recommendation, level of evidence C), particularly those without overt disease. Genetic testing in probands fulfilling only one minor criterion is not recommended. A family background and identification of a pathogenic mutation has been demonstrated in up to 50%, while in the remaining probands, an underlying familial disease with incomplete penetrance cannot be excluded. The most common mutations are found in PKP2 (80% of mutations in the Dutch and Northern American cohorts) and DSP (39% in the Italian cohort)^[80], followed by DSG2^[8,47,62,136,137]. It should be kept in mind that molecular genetic testing may only support a clinical diagnosis or suspicion. A negative test does not rule out AVC, because other causal genetic mutations and unknown environmental factors may also cause the disease^[47,138]. Pathogenic mutations do not make a diagnosis of AVC itself, as multiple sources of diagnostic information such as ECG

changes, ventricular arrhythmias and ventricular abnormalities have to be considered^[56]. Yet, the identification of pathogenic mutations may be useful in the differential diagnosis of AVC and phenocopies, such as myocarditis, idiopathic RVOT tachycardia, DCM, muscular dystrophies, IMF or sarcoidosis^[35]. Cascade genetic screening of relatives may offer another strategy to serial non-invasive cardiovascular evaluation of family members. Current guidelines^[1,87] do not recommend genetic testing for risk stratification and therapeutic decision making in AVC because study results regarding the ability of genotyping to detect malignant mutations associated with an increased susceptibility to potentially lethal arrhythmias have been conflicting^[8,62,64,90,139]. Recent large scale studies^[8,62] indicate an association between positive mutation carrier status and early disease onset. Thus, genotyping of younger family members should strongly be encouraged. This might be particularly important for patients carrying digenic or compound heterozygote mutations that are reported in up to 18% of the AVC population studied and have been associated with a stronger phenotype^[1]. Issues such as the availability of genetic counselling in a multidisciplinary setting^[140], low-probability mutations^[136], genetic testing for “low-probability” AVC, psychological repercussions of young patients, and costs need to be considered before performing genetic screening^[75].

DIFFERENTIAL DIAGNOSIS

Idiopathic RVOT-VT is a major non-hereditary differential diagnosis that has to be distinguished from ARVC/D. This is often demanding, particularly in the early stages of AVC^[141]. RVOT-VT is not associated with structural heart disease and thus has a more benign course. Its etiology is unclear, although in one study a somatic point mutation in the inhibitory G protein Gai2 was identified by EMB from the arrhythmic focus^[142]. In RVOT-VT, 12-lead surface ECG and SAECG are normal during sinus rhythm. It is characterized by repetitive monomorphic VT of a single morphology with LBBB morphology and an inferior axis. Similar VT morphologies can be found in patients with ARVC/D. 12-lead ECG scoring systems to differentiate both types of VT have recently been proposed^[108]. In ARVC/D the duration of the QRS complex during VT is usually longer (≥ 120 ms in lead I)^[143]. Notching of the QRS and precordial transition in lead V6 may exclusively be seen in ARVC/D^[144]. RVOT-VT is difficult to induce by programmed ventricular stimulation during EPS, particularly in the absence of isoproterenol^[87]. It responds well to beta-blockers or verapamil and ablation after successful mapping is usually curative. EAM demonstrates normal voltages. CPVT is caused by mutations in the RyR2 gene, which has also been described in ARVC/D subtype 2. CPVT is characterized by effort-induced polymorphic VT in patients with structurally normal hearts. Genetic analysis, a positive family history, EAM and EMB can help to differentiate AVC and regional myocarditis^[14]. Myocardial involvement in sarcoidosis can mimic ARVC/D and the

current TFC do not reliably distinguish between them. In a prospective study of patients with suspected ARVC/D, evaluated by a protocol including EMB, a surprisingly high incidence (15%) of cardiac sarcoidosis was verified^[145]. Sarcoidosis with cardiac involvement thus always needs to be considered, particularly if respiratory and systemic symptoms, high-grade atrioventricular conduction block, and no family disease are present. Similar clinical presentations and imaging findings can pose a challenge in the absence of histological diagnosis. Features favoring cardiac sarcoidosis include early septal involvement, reduced LV function, a wide QRS during VT, right-sided apical VT and more inducible forms of monomorphic VT^[146]. Diagnosis is usually confirmed by EMB^[147]. In patients who survive SCD, ischemic heart disease and an anomalous origin of the coronary arteries have to be excluded. DCM is particularly difficult to distinguish from non-classic forms of AVC. Palpitations, (pre)syncope and ventricular arrhythmias are present at an early stage in AVC, often in the absence of gross structural abnormalities, which is usually not the case in DCM. Subepicardial LGE on CMR, particularly in the posterobasal LV wall, also favors AVC^[36]. Atrioventricular conduction block is more common in DCM, but mutations in lamin A/C can cause AVC with conduction defects^[148]. Bs may mimic ARVC as RV conduction delay has been demonstrated in both and recently a genetic overlap between these two entities has been proposed^[94,149]. The presence of gross structural abnormalities favors AVC and mutations in SCN5A are very rare in AVC. Further differential diagnoses include RV infarction, pulmonary hypertension, congenital left-to-right shunts, Chagas disease and Uhl's disease (congenital hypoplastic RV).

DISEASE COURSE AND PROGNOSIS

Although AVC is a progressive disease, the individual disease course can vary considerably. The mortality rate is currently estimated to be around 1%-3% per year. In one study, after 8 years of mean follow-up, total mortality was approximately 20% and the mean age at death 54 ± 19 years. Most patients died of progressive heart failure (59%) and VTA (29%)^[150]. Embolic stroke may lead to death in a smaller proportion of patients.

AVC occurs in four phases^[2]: (1) concealed phase, during which patients are asymptomatic and structural abnormalities are absent or subtle. Nevertheless, AVC can present with SCD as the primary manifestation; (2) occurrence of symptomatic arrhythmias; (3) early heart failure symptoms; and (4) end-stage heart failure necessitating a ventricular assist device or cardiac transplantation. One study has shown that 7% of AVC patients received cardiac transplantation after a mean follow-up period of 10 years and severe LV involvement is often present in this population^[7]. Strenuous physical activity often leads to early disease manifestation and rapid disease progression. Young competitive athletes with AVC have a 5-fold increased risk of SCD compared to non-athletes and

identification of affected athletes by pre-participation screening has substantially reduced mortality in this cohort^[64,151]. Interestingly, in one study, mutation-carrying female relatives were less frequently affected than male relatives. This has been interpreted as prevention of apoptosis in cardiac myocytes by estradiol but could also be related to more life-long physical activity in men^[152].

RISK STRATIFICATION

SCD in patients with AVC is difficult to predict and often occurs without alarming symptoms. The only reliable strategy for SCD prevention is the implantation of an ICD, with an annual incidence of appropriate ICD interventions among AVC patients of 5%-22%, demonstrating its importance for these patients. Thus, in secondary prevention after aborted SCD, VF or sustained VT, ICD implantation is recommended^[87,147]. Besides aborted SCD, VF and sustained VT, other potential risk factors for SCD or appropriate ICD therapy (a surrogate marker for SCD) have been suggested: (1) syncope (DARVIN 2 study)^[93]; (2) left ventricular dysfunction^[7,56,153]; (3) young age at presentation^[62,63,67] and young age per se^[47,64]; (4) RV structural abnormalities fulfilling 2010 TFC^[47,154]; (5) severe tricuspid regurgitation^[7]; (6) particular genetic variants^[8,72]; (7) presence of non-sustained VT^[155]; (8) male gender^[79]; (9) proband status^[79]; (10) frequent PVC^[79]; and (11) presence of precordial T-wave inversions^[79].

It is important to recognize that the use of appropriate ICD therapy due to sustained VT or VF as a surrogate for SCD can result in an overestimation of this endpoint. Whether in the absence of arrhythmic syncope or significant ventricular arrhythmias the other potential risk factors are consistently related to an adverse arrhythmic outcome and require prophylactic ICD therapy remains to be determined by future studies. Of note, young patients may suffer from neurocardiogenic syncope, making differential diagnosis difficult and its prognostic value elusive. T-waves in the precordial and inferior leads often become negative with progression of AVC and a greater extent of precordial negative T-waves are associated with more severe RV dilation and dysfunction^[100]. Recently, the Johns Hopkins group found that 88% of patients with documented sustained VTA exhibited an abnormal ECG. A total of 122 (84%) subjects demonstrated T-wave inversions in the precordial leads with 97 of them extending to lead V3 and beyond, while depolarization abnormalities such as epsilon waves were present only in a minority of patients^[156]. The same group found that the presence of T-wave inversions in ≥ 3 precordial ECG leads was an independent predictor of adverse events during follow-up^[79]. An Italian group has also demonstrated a link between the extent of negative T-waves and ventricular arrhythmic events during follow-up^[157]. Although a class II b recommendation, the role of EPS with programmed ventricular stimulation for risk stratification in AVC is less well established and conflicting data about its prognostic significance exist^[45,64,90,158]. Differ-

ences in the studied patient population may be influenced by disease severity^[159] and differences in study design may have led to discrepant results. A positive family history of SCD in asymptomatic patients does not seem to increase their individual risk for lethal arrhythmias. Guidelines do not support genetic testing for risk stratification in AVC^[1] and genotype-phenotype correlation studies so far have not consistently been able to show that genotyping is able to detect mutations specifically associated with an increased susceptibility to life-threatening arrhythmic events. However, recent data indicates that certain pathogenic mutations (*e.g.*, plakoglobin in Naxos disease, RyR2 and TME-43) may increase the risk for SCD^[8,62,67]. These preliminary results have to be confirmed in larger studies and more precise risk stratification tools for asymptomatic patients are needed. Novel imaging modalities such as strain and three-dimensional echocardiography could help to further improve risk stratification^[160].

Based on the available data from observational studies, we suggest classifying patients into three risk categories^[79,161]: (1) high risk: aborted SCD, sustained VT and VF, arrhythmic syncope; (2) moderate risk: non-sustained VT, severe structural abnormalities of RV and/or LV, presence of cardiac symptoms, ≥ 3 leads with T-wave inversions, frequent PVCs (*i.e.*, > 760 PVC/24 h Holter) and severe disease onset age < 35 years; and (3) low risk: asymptomatic family members (also despite a positive family history of SCD), < 10 PVC/24 h Holter.

The risk factors listed here have focused largely on patients with right-dominant disease. Prognostic factors in non-classic disease still remain elusive. Patients should be astute for symptoms. Dynamic T-wave inversions, ST segment elevation and myocardial biomarker release mimicking myocardial infarction should alert the treating physicians to think of a “hot phase” of AVC. Clinical evaluation starting at age 10-12 is suggested for all first- and second-degree relatives of AVC index patients until age 60^[140]. If SCD occurs at age < 35 , a full postmortem autopsy by an expert cardiac pathologist including molecular autopsy screening for genetic variants should be performed.

THERAPY

Physical activity restriction

It is a general consensus that strenuous physical activity should be avoided in symptomatic patients with AVC. There is no consensus that physical activity should be avoided in asymptomatic healthy gene carriers. A recent study has shown that endurance exercise and frequent exercise increase the risk of VT/VF and heart failure in patients, but also in healthy family members carrying a pathogenic desmosomal mutation, supporting exercise restriction for these patients^[82]. We prudently advise all symptomatic patients and healthy gene carriers to refrain from practicing competitive sports and strenuous physical exercise, not only for reducing the risk of ventricular arrhythmias, but also to prevent disease onset

and progression.

Pharmacological therapy

Beta-blockers, amiodarone and sotalol can be effective for treatment of sustained VT or VF in patients with AVC. However, they have no proven prognostic benefit such as ICD therapy. Wichter *et al.*^[107] proved that sotalol is highly effective to suppress VT by programmed ventricular stimulation with an efficacy of 68% and 83%, respectively, but had no effects on prognosis and SCD. Amiodarone was not superior to sotalol in this study and is not considered first-line therapy by many clinicians because of frequent side effects during long term therapy, particularly in young patients. However, recent data from the Northern American ARVC registry demonstrated amiodarone to confer the greatest efficacy in preventing ventricular arrhythmias when compared to sotalol or beta-blockers. However, mean sotalol doses were lower than in the study from Wichter *et al.*^[107] and only ten patients were treated with amiodarone in the American study. In clinical practice, beta-blockers, sotalol or amiodarone are often used as an adjunctive therapy to reduce arrhythmia burden in patient with an ICD and amiodarone is sometimes combined with beta-blockers in order to reduce sympathetic tone and mechanical wall stress^[162]. Co-administration of sotalol and amiodarone is not recommended due to QT interval prolongation. Hiroi *et al.*^[163] suggest that carvedilol may control arrhythmias and improve LV function in some patients with biventricular AVC. Calcium antagonists such as verapamil and mexiletin may be effective in some patients to suppress VT but data is anecdotal. If heart failure occurs, standard therapy with beta-blockers, angiotensin converting enzyme-inhibitors and a diuretic should be established, although there are no specific studies in patients with AVC^[46]. Brain natriuretic peptide, C-reactive protein, IL-1 β and TNF- α as surrogate biomarkers for disease activity, inflammation and prognosis have been advocated in AVC but await further validation^[3,58,164]. AVC patients at later stages have an increased risk for thromboembolism^[43]. The annual incidence of thromboembolic complications, including pulmonary embolism, RVOT thrombosis and cerebrovascular events, was 0.5% in a retrospective study of 126 patients followed up for a mean period of 99 ± 64 mo^[56]. Anticoagulation is often started by clinicians in the presence of severe ventricular dilation, dysfunction and aneurysm, although existing studies do not support prophylactic use in those with RV aneurysms. Data for the non-classic subtypes are lacking.

Implantable cardioverter-defibrillator

According to the ACC/AHA/HRS 2008 Guidelines for Device-Based Therapy of Cardiac Rhythm Abnormalities and its recent update^[165,166], ICD implantation is indicated in patients with structural heart disease who have experienced a sustained VTA (secondary prevention, Class I indication). It is also stated that ICD implantation is reasonable in AVC patients who have at least one

risk factor for SCD (II A indication, level of evidence C). Thus, ICDs constitute a cornerstone for those patients and can prolong survival in this population. In fact, a large number of studies has demonstrated that patients with AVC who undergo ICD implantation have a high likelihood for appropriate ICD therapies^[167]. However, many questions remain regarding AVC patients and their relatives who are at low to moderate risk for SCD. In these patients, a lifelong risk for lethal arrhythmias has to be weighed against the complication rates of ICDs, inadequate interventions (up to 24% within 5 years), psychological burden and economic costs of this therapy. However, complication rates seem to have declined since the use of third- or fourth-generation defibrillators. Active and young patients are at particular risk of lead displacement and inappropriate discharges for sinus tachycardia, including painful shocks and multiple invasive procedures. Thus, indiscriminate device implantation cannot be endorsed. Instead, reliable risk stratification is of paramount importance. An ICD with dual chamber detection algorithms may be wise in young patients to discriminate VT or SVT from sinus tachycardia. The use of antiarrhythmic agents can also reduce the number of inadequate interventions due to supraventricular tachyarrhythmias. Furthermore, programming of higher VT/VF cut-offs and longer detection intervals can avoid inappropriate ICD shocks^[168]. Complications of ICD therapy include a risk for perforation caused by thinning of the RV wall, lead dislodgement, R wave under-sensing and high pacing thresholds. As patients are young and mobile, these risks need particular consideration, although in one study, short and long-term risks of ICD therapy were similar to patients without AVC^[45].

In our clinical routine, we recommend ICD implantation for all AVC patients who have experienced a sustained VTA but we also carefully evaluate ICD implantation for primary prevention in probands and family members without documented sustained VTA. Therefore, we evaluate whether a particular patient (1) has high-risk features for SCD during follow-up (see list above), (2) whether the patient is willing to take his medication regularly and to stop competitive sports (*i.e.*, competitive individual events like triathlon or participation in a competitive sports team), and (3) the patient's preferences. Our threshold for ICD implantation is higher in family members and asymptomatic patients owing to the fact that previous studies have consistently shown that family members are at lower risk of experiencing sustained VTA. A possible explanation for this finding is that diagnosis occurs earlier in the disease course and once diagnosed, family members are encouraged to give up competitive sports. However, more data obtained from different well characterized AVC cohorts are necessary to assist clinicians in guiding ICD therapy.

Catheter ablation

Catheter ablation was first applied to treat drug-resistant VT. The application of direct current (DC) termed fulgura-

tion, used DC from a defibrillator to burn myocardial sites responsible for abnormal ventricular activation. The electric voltage was directly delivered through a catheter to the origins of VT. However, this procedure was associated with a significant risk of complications and thus rapidly abandoned. Currently accepted indications for radiofrequency catheter ablation in patients with AVC include drug-refractory VT or incessant VT with frequent ICD shocks. It should be kept in mind that, unlike in patients with idiopathic VT where catheter ablation is curative, catheter ablation in patients with AVC can only improve quality of life by decreasing the number of VT episodes and PVCs^[169]. Catheter ablation can follow a trial of beta-blocker therapy and antiarrhythmic therapy. In some patients who do not wish long-term therapy with beta-blockers, sotalol and particularly amiodarone, catheter ablation can be performed as first line therapy. Elimination of clinical tachycardia can relieve symptoms but may not prevent SCD.

Over the last years, mapping and ablation techniques have made outstanding progress and nowadays include activation, pace and entrainment mapping during VT and substrate-based ablation using EAM that can be performed *via* an endocardial and epicardial approach^[170]. Substrate-based ablation of PVCs and VT is particularly important when conventional mapping during tachycardia is not possible due to hemodynamic instability or multiple VT morphologies^[171]. Although the initial approach involved extensive mapping to identify critical zones of slow conduction during VT, this approach has recently been replaced by a substrate-based approach. Preliminary studies have shown promising results regarding safety, arrhythmia-free survival and reduction of ICD discharges, particularly if an endocardial and epicardial approach are combined^[128-131]. In one recent study from the Johns Hopkins cohort, the overall freedom from VT was 47%, 21% and 15% at 1, 5 and 10 years, respectively. Following epicardial VT ablation, the cumulative freedom from VT was 64% and 45% at 1 and 5 years. Of note, the VT burden decreased from a median of 0.16 VT episodes per month pre ablation to 0.08 episodes per month post ablation^[172]. Mid-term and long-term success and safety of these methods have to be demonstrated in future studies with larger cohorts.

Surgical methods

Total surgical electrical RV disconnection carries an important risk of postoperative RV failure and has been practically abandoned^[173]. If severe therapy refractory heart failure occurs, ventricular assist devices or heart transplantation have to be considered for isolated LV or biventricular failure and less frequently isolated RV failure. Some authors suggest that right heart catheterization should be performed in all cases with suspected severe RV dysfunction. If increased filling pressures suggest a Fontan-type physiology, the patient may be considered for heart transplantation^[174].

CONCLUSION

During the last three decades, our understanding of AVC from a developmental RV dysplasia with substitution by adipose tissue has remarkably changed to a mostly inherited polygenic disease of the intercalated disc with a broad phenotypic spectrum. Although AVC predominantly affects the RV, non-classic forms affecting the LV or both ventricles are increasingly recognized. A hallmark is the early propensity to ventricular arrhythmias associated with SCD at a young age. Enormous progress in unravelling the genetic and molecular basis of this complex disease, in which environmental factors seem to play a pivotal role, has been made in the last years. While progress in imaging and device therapy has facilitated clinical diagnosis and prevention of SCD, today's challenges include discovery of novel genetic and environmental factors, early detection of asymptomatic patients, improved risk stratification, catheter ablation strategies and causal therapies to cure the disease^[175]. Multicenter, large, prospective follow-up studies are planned to improve our understanding of the complex underlying molecular mechanisms of AVC, which may facilitate diagnosis, risk stratification and causal therapy.

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High-sensitivity cardiac troponins in everyday clinical practice

Johannes Mair

Johannes Mair, Department of Internal Medicine III, Cardiology and Angiology, Innsbruck Medical University, A-6020 Innsbruck, Austria

Author contributions: Mair J solely contributed to this paper.
Correspondence to: Johannes Mair, MD, Associate Professor, Department of Internal Medicine III, Cardiology and Angiology, Innsbruck Medical University, Anichstrasse 35, A-6020 Innsbruck, Austria. johannes.mair@i-med.ac.at

Telephone: +43-512-50481314 Fax: +43-512-50422767

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Abstract

High-sensitivity cardiac troponin (hs-cTn) assays are increasingly being used in many countries worldwide, however, a generally accepted definition of high-sensitivity is still pending. These assays enable cTn measurement with a high degree of analytical sensitivity with a low analytical imprecision at the low measuring range of cTn assays (coefficient of variation of < 10% at the 99th percentile upper reference limit). One of the most important advantages of these new assays is that they allow novel, more rapid approaches to rule in or rule out acute coronary syndromes (ACSs) than with previous cTn assay generations which are still more commonly used in practice worldwide. hs-cTn is also more sensitive for the detection of myocardial damage unrelated to acute myocardial ischemia. Therefore, the increase in early diagnostic sensitivity of hs-cTn assays for ACS comes at the cost of a reduced ACS specificity, because more patients with other causes of acute or chronic myocardial injury without overt myocardial ischemia are detected than with previous cTn assays. As hs-cTn assays are increasingly being adopted in clinical practice and more hs-cTn assays are being developed, this review attempts to synthesize the available clinical data to make recommendations for their everyday clinical routine use.

reserved.

Key words: Cardiac troponin; High-sensitivity; Diagnosis; Acute myocardial infarction; Acute coronary syndrome; Review

Core tip: High-sensitivity cardiac troponin (hs-cTn) assays enable cardiac troponin measurement with a high degree of analytical sensitivity with a low analytical imprecision at the low measuring range. One of the most important advantages of these new assays is that they allow novel, more rapid approaches to rule in or rule out acute coronary syndromes (ACSs). The increase in early diagnostic sensitivity of hs-cTn assays for ACS comes at the cost of a reduced ACS specificity, because more patients with other causes of acute or chronic myocardial injury without overt myocardial ischemia are detected than with previous cTn assays.

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INTRODUCTION

Cardiac troponin I (cTnI) and cTnT are the biomarkers of choice for the diagnosis of myocardial damage, because they are the most sensitive and cardiac-specific biomarkers currently available^[1,2]. Recommendations for the use of cTn measurement in acute cardiac care^[1] and practical clinical considerations in the interpretation of cTn elevations^[2] have been published recently. Over the years the analytical sensitivity of cTn assays has been continuously improved, and more recently a new generation of cTn assays, *i.e.*, the high-sensitivity (hs)-cTn assays, have been introduced into routine clinical practice^[3]. It is important to note, that these assays measure the same analyte as previous assay generations but with

substantially improved analytical sensitivity and assay precision at the low measuring range^[3-6]. It is also important to note because of discrepancies in routine use^[7,8], that, regardless of how assays are named by manufacturers, hs-cTn assays should be only designated as hs-cTn assays, if the below listed analytical characteristics are met by an assay also in routine use together with publication of its hs analytical characteristics in peer-reviewed literature^[7,8].

From a clinical perspective it has been noted that the improved analytical performance of hs-cTn assays also increased their clinical ability to detect small amounts of myocardial damage and to precisely identify small differences in cTn concentrations in serial testing compared with previous cTn assay generations^[8]. It is expected that hs-cTn assays, if used appropriately, will improve both early diagnosis and short and long-term risk stratification. In this review recommendations for the clinical interpretation of hs-cTn test results are proposed based on the currently available clinical evidence, and it is also indicated where sufficient clinical data are still lacking.

ANALYTICAL CHARACTERISTICS OF HS-CTN ASSAYS

The analytical characteristics of hs-cTn assays are summarized in Table 1. The analytical lower limit of detection (LoD) is in the range of single digits of ng/L or even below^[7-11]. Therefore, it is recommended that hs-cTn assay results are reported as ng/L (= pg/mL), and cTn values below the LoD should not be reported as numbers^[8]. hs-cTn assays must have high precision in routine use at lower concentration ranges with total analytical coefficient of variation (CV) < 10% at the 99th percentile concentration of the reference population, which is the recommended upper reference limit (URL). Despite increased analytical sensitivity hs-cTn assay must maintain analytical specificity for the detection of cardiac troponin isoforms. There have not been reports of major analytical interferences with hs-cTn assays, but they are possible and thorough evaluations of possible analytical interferences is needed before approval for routine use^[7,8]. In contrast to conventional cTn assays, hs-cTn assays permit measurement of cTn concentrations in a significant proportion of apparently pathology-free individuals, which favours a precise calculation of the URL^[1,7]. There is still no consensus on a specific percentage of detectable cTn concentrations in the reference population which is required for the label hs as long as all the other criteria are fulfilled, but usually > 50% are recommended^[7]. There are reports on sex-specific URLs which are higher for men than women for hs-cTn assays including the already commercially available hs-cTnT and hs-cTnI assays from Roche and Abbott Diagnostics^[3,5,7-11], and it may turn out that sex-specific URLs should be used in routine as well. The underlying mechanisms for cTn release from normal hearts are still uncertain and remain to be established. Since analytical interferences can be ruled out^[3,5,10], a constant limited turnover of cardiomyocytes appears to be present in normal hearts as well.

Table 1 Analytical characteristics of high-sensitivity cardiac troponin assays

The analytical lower limit of detection is in the range of single digits of ng/L and is markedly lower than the upper reference limit
Hs-cTn assays have high precision in routine use at lower concentration ranges with analytical CV < 10% at the 99 th percentile concentration of the reference population
Hs-cTn assays enable detection of cTn in a significant proportion of the reference population, thereby allowing for a more accurate calculation of the 99 th percentile URL with its 95% confidence interval
Hs-cTn assays must be highly specific for the detection of cardiac cTn isoforms

Hs-cTn: High-sensitivity cardiac troponin; CV: Coefficient of variation; URL: Upper reference limit.

EARLY DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION

Hs-cTn assays detect cTn release at an earlier time point than the previous generations of cTn assays leading to an improved early sensitivity for acute myocardial infarction (AMI) diagnosis within 3 h of presentation^[12-15]. Most but not all studies demonstrated a higher diagnostic accuracy of hs-cTn assays for early AMI diagnosis when compared to previous cTn assay generations on admission to the emergency department^[16]. However, scrutiny is needed when evaluating studies on this topic as differences between assays often have been overstated by use of different medical decision limits for the older and newer cTn assays, e.g., 10% CV concentration limit *vs* 99th percentile URL. This leads to apparent higher specificity and lesser sensitivity with non hs-cTn assays and magnifies the differences in early sensitivities at patient presentation observed with the hs-cTn assays^[16]. However, guidelines recommend the use of the URL as a medical decision limit even when it cannot be measured with a CV of < 10%^[17]. Thus early sensitivities must be compared by using the 99th percentile URL as a medical decision limit for standard and hs-cTn assays. In addition, some patients may not have AMI diagnosed because their standard cTn values do not increase above the cut-off value but do so with the hs-cTn assay. Thus, a significant number of patients with unstable angina may migrate from that designation to the AMI category if reclassified using the hs-cTn test results. Studies of the diagnostic performance of hs-cTn assays in more heterogeneous populations are also still needed because most present studies have been done in pre-selected emergency department populations presenting with cardiac symptoms or chest pain unit populations. Study design influences the sensitivity and the specificity of cTn, the optimal blood sampling regimens, and optimal decision limits for absolute or relative changes in serial testing. Statistical analyses are also heterogeneous. Most studies determine optimal decision limits according to receiver operating characteristic curve analysis which weighs sensitivity and specificity equally, while others have optimized cut-off values for specificity. The selection of criteria for change limits for AMI diagnosis will also differ depending on whether there is

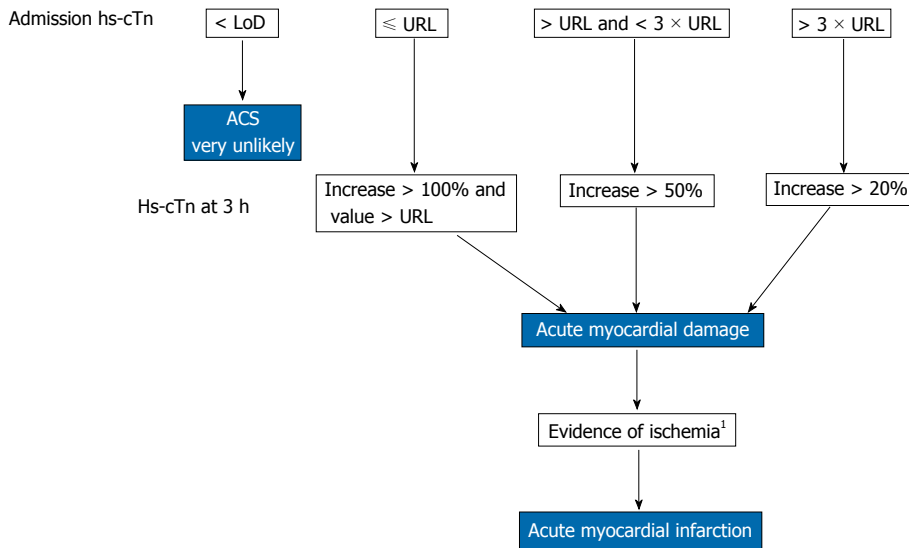


Figure 1 Algorithm for the rapid evaluation of clinically suspected acute myocardial infarction with high-sensitivity cardiac troponin testing. This algorithm is based on best current knowledge and may have to be modified with upcoming new data. This approach at least guarantees that the changes will be above the analytical and biological variation. It is important to note that hs-cTn changes over a 3 h period in patients presenting late after AMI onset may be less than 20%. For hs-cTnT some studies favour absolute changes over relative concentration changes. ¹Evidence of acute myocardial ischemia by new ECG changes and/or new imaging corroborations. Hs-cTn: High-sensitivity cardiac troponin; URL: 99th percentile upper reference limit of healthy controls; ACS: Acute coronary syndrome; LoD: Lower limit of detection; AMI: Acute myocardial infarction; ECG: Electrocardiogram.

a need for high specificity at the cost of lower sensitivity or increased sensitivity at the cost of lower specificity. Clinicians must be aware of this trade off in evaluating individual patients. For all these reasons, the pooling of study data from the literature is currently problematic.

Clinically relevant hs-cTn assay concentration changes in serial testing

Key to the use of hs-cTn assays is the need to evaluate cTn kinetics with serial testing in the clinical evaluation of chest pain patients^[18,19]. At least two measurements of hs-cTn test results to verify a kinetic pattern are required to comply with the Universal Definition of Myocardial Infarction^[20]. Even in patients with increased hs-cTn values a significant change must be documented by serial measurements.

In general, most AMI patients have substantial and obvious changes in hs-cTn values. It must be emphasized that dynamic changes are not specific for AMI but are rather indicative of acute myocardial damage. An algorithm for the use of hs-cTn serial measurements for the evaluation of AMI in patients presenting with symptoms suggestive for an acute coronary syndrome (ACS) based on the currently available clinical data is shown in Figure 1. Previous recommendations on change criteria just considered analytical variation and advocated based on a total CV < 10% any change in serial testing of > 20% to be significant^[21]. The precision necessary to implement this approach is not present within the reference range for hs-cTn assays either^[11]. In addition, biological variation needs to be considered. Changes of hs-cTn measurements near the 99th percentile URL must exceed conjoint analytical and biological variation to be of clinical significance. This is done by calculation of the so-

called reference change values (RCV). Such values can be calculated only for reference individuals, but the theory of biological variation postulates the same process in patients with disease. These calculated RCV values are assay and analyte specific and must be obtained separately for each commercially available hs-cTn assay. For many assays, short-term RCVs are in the 40%-60% range^[22-24], although one report has values as high as 86%^[25]. Data on short- and long-term variation of hs-cTn concentrations in clinically stable patients with chronic cardiac diseases are very limited^[26], but the reported variation is in the range of healthy individuals. A recently published study evaluating serial changes using a pre-marketing version of the Abbott® hs-cTnI assay in pre-selected chest pain unit patients, suggested that increases above the 99th percentile URL with relative increases of > 250% over a 3 h period in patients with baseline values < URL and increases > 50% with modestly increased baseline values optimize specificity for the diagnosis of AMI^[15]. However, AMI diagnosis in this study was based on clinical criteria and an increase in a conventional local cTnI assay > 99th percentile URL with a > 20% change over a 6 h period. As expected, higher cTnI sensitivities were found at lower percentage changes.

Whether the diagnostic performances of percentage change differ from an absolute change of cTn concentrations, has been tested with the hs-cTnT assay in recent clinical studies^[27,28]. It has been described at hs-cTnT values below or close to the 99th percentile URL that an absolute increase of hs-cTnT values (*e.g.*, > 7 ng/L over 2 h) is superior to a relative percentage changes from baseline. Other hs-cTn assays may require different metrics, because data on absolute changes in serial testing are assay specific. Undetectable hs-cTn ruled out ACS with a

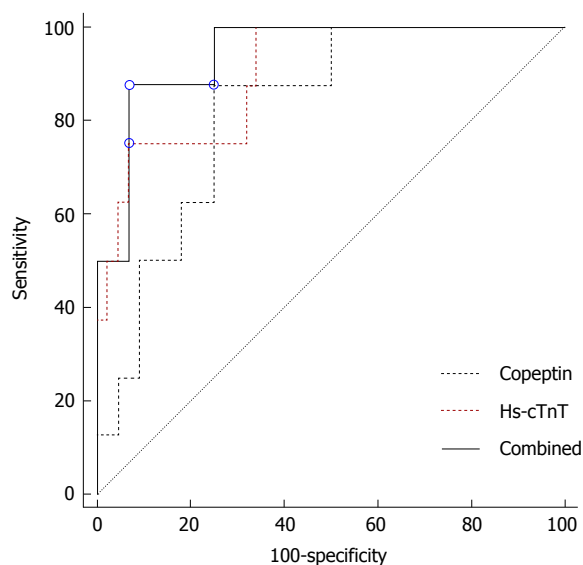


Figure 2 Diagnostic performances of high-sensitivity troponin T and copeptin for the diagnosis of acute myocardial infarction in chest pain patients. Own unpublished results, the area under receiver operator characteristic curves of the combination of copeptin with hs-cTnT (0.94) was not significantly different from the area under hs-cTnT curve (0.90). The worthless test is indicated as reference line. Hs-cTnT: High-sensitivity cardiac troponin T.

negative predictive value > 99% on ED admission^[15,16].

Timing of hs-cTn measurements in serial testing

According to the recent European guideline for the management of ACS, blood samples should be obtained at the time of presentation and 3 h after admission when using hs-cTn assays^[19]. There is recent evidence suggesting that many patients with an AMI can be reliably identified within 3 h after admission with close to 100% sensitivity and negative predictive value using a hs-cTn assay, which indicates that observation time in the emergency department may be reduced for the rule out of AMI^[12-15]. However, most of these studies based the diagnosis of AMI on the prior less sensitive cTn assays and ignored AMI only detected with hs-cTn assays. Thus, if the clinical situation is ambiguous and the pre-test likelihood of disease is high, additional subsequent sampling (*e.g.*, at 6 h and even beyond) is still necessary in individual patients.

Myocardial infarction after percutaneous coronary interventions or aortocoronary bypass grafting

There are still no data on hs-cTn decision limits in these clinical settings. In acute percutaneous coronary interventions (PCI) or nowadays rarely performed acute coronary artery bypass grafting (CABG) for evolving AMI acute myocardial damage is caused by AMI itself and the potential additional myocardial damage caused by PCI or CABG cannot be differentiated from cTn release caused by ongoing AMI. In elective PCI or CABG, by contrast, baseline cTn values are usually within the normal range and potential myocardial damage caused by these interventions can be reliably detected by hs-cTn measurements. However, in these elective patients hs-cTn decision limits for periprocedural AMI are also still

not available. Thus, only the limits recommended by the universal definition of AMI can be currently used^[20], *i.e.*, increase > 5-times URL after PCI and > 10-times URL after CABG. However, these limits are still very controversially discussed in the communities of interventionists and cardiac surgeons, because it appears from the available data that periprocedural cTn increases in clinically uncomplicated patients must be substantially higher to be of prognostic significance^[29].

DO WE NEED ADDITIONAL BIOMARKERS FOR AMI DIAGNOSIS WHEN HS-CTN ASSAYS ARE USED?

The most recently advertised markers for the early diagnosis of AMI are heart-type fatty acid binding protein (H-FABP) and copeptin. However, in the vast majority of studies these markers were compared only with previous, less sensitive cTn assays and comparative data with hs-cTn assays are still limited.

H-FABP

Despite its name this protein is not a cardiac-specific marker as it is also expressed, although in much lower amounts, in several other tissues. It is cleared by the kidneys and thereby increased in case of renal failure^[30]. H-FABP increases rapidly in ACS^[31], but more recent data do not support a benefit when combined with hs-cTn^[15,32].

Copeptin

Copeptin is the 39 amino acids long c-terminal part of pro-arginine-vasopressin and a stable surrogate marker of vasopressin secretion^[33]. It is a marker of stress^[33] and has been proposed for early AMI diagnosis on emergency department admission^[34]. More recent data do not support a benefit when combined with hs-cTn (Figure 2)^[15,32].

In summary, when hs-cTn assays are used instead of standard cTn assays both H-FABP and copeptin do not add to the early diagnosis of AMI, particularly, if the LoD is used as an AMI rule-out limit for hs-cTn in chest pain patients. However, in case of point-of-care testing where the criteria for hs are very difficult to be fulfilled for cTn assays a combination with these markers may be useful.

DISEASES WITH POTENTIAL HS-CTN ELEVATIONS OTHER THAN AMI

Given the high frequency of detectable and slightly elevated hs-cTn values in the community^[35-37], especially in patients with cardiovascular comorbidities, it is important to note that an increased hs-cTn concentration alone is not sufficient to make the diagnosis of AMI^[20]. hs-cTn increases must, therefore, be interpreted in relation to the clinical presentation (Table 2). Thus, a recent publication suggested that it may be advisable to use a higher cut-point (about 3-fold the 99th percentile URL) as a decision

Table 2 Elevations of high-sensitivity cardiac troponin in the absence of significant coronary artery disease

Acute myocardial damage related to secondary myocardial ischemia (AMI type 2)	Tachycardia or bradycardia (<i>e.g.</i> , rapid pacing during transcatheter aortic valve replacement) Aortic dissection with involvement of coronary ostia Severe aortic valve stenosis Hypertrophic cardiomyopathy Hypo- or hyper-tension (<i>e.g.</i> , hemorrhagic shock, hypertensive emergency) Acute heart failure without significant concomitant CAD Severe pulmonary embolism or pulmonary hypertension Coronary vasculitis, <i>e.g.</i> , systemic lupus erythematosus Coronary endothelial dysfunction (spasm) without significant CAD, <i>e.g.</i> , cocaine abuse Coronary embolism
Acute myocardial damage not related to myocardial ischemia	Cardiac contusion Cardiac incisions with surgery Radiofrequency or cryoablation therapy for arrhythmias Rhabdomyolysis with cardiac involvement Myocarditis Cardiotoxic agents, <i>e.g.</i> , anthracyclines, CO poisoning, severe burns affecting > 30% of body surface
Indeterminate or multiform group	Apical ballooning syndrome Renal failure Severe acute neurological diseases, <i>e.g.</i> , stroke, trauma Infiltrative diseases, <i>e.g.</i> , amyloidosis, sarcoidosis Extreme exertion Sepsis Acute respiratory failure Frequent defibrillator shocks
Analytical interferences	Rare, <i>e.g.</i> , by high titres of auto- or hetero-philic antibodies

AMI: Acute myocardial infarction; CO: Carbon monoxide; CAD: Coronary artery disease.

limit for AMI in > 70 year-old patients^[38]. However, it is likely that most of elevations in the elderly are caused by comorbidities. Thus, the use of higher cut-off values decreases early sensitivity for AMI in older patients without comorbidities. Regardless of the cut-off value used, the critical distinction that remains to be made is to determine whether there is a significant rising pattern of hs-cTn values in serial testing as an indicator of acute myocardial damage. Thus, clinical judgement still remains essential.

With hs-cTn assays, elevations above the 99th percentile URL are common in patients with structural heart disease (Table 2), including patients with stable coronary artery disease^[39,43]. In patients with putative stable angina, a hs-cTnT value > 99th percentile URL is found in 37% of those with coronary plaques that are thought to be more labile or vulnerable^[39,40]. In stable heart failure patients, the median concentration for hs-cTnT is 12 ng/L, which is very close to the 99th percentile URL of 14 ng/L for this assay^[42,43]. However, regardless of the cause, elevations of hs-cTn values are associated with an adverse clinical outcome in most clinical conditions, as in patients with AMI, stable CAD, heart failure, pulmonary embolism or chronic pulmonary arterial hypertension^[35,37,39,41-45].

Cardiac specificity of cTnT vs cTnI

A recent report again raised concerns regarding the cardiac-specificity of the current generation cTnT assay in patients with chronic skeletal muscle disorders due to potential reexpression of cTnT isoforms or expression of an immunoreactive protein in skeletal muscle myopathies. In patients without evidence of myocardial injury increases of creatine kinase MB (CKMB) iso-

zyme and cTnT without concomitant increases in cTnI were found^[46]. A potential release of cTnT from skeletal muscle with normal cTnI in patients with chronic skeletal muscle damage is also highlighted by an own case in whom we measured cTnT and cTnI with hs assays (Figure 3). The most cardiac-specific marker in this rare patient population with chronic skeletal muscle damage (*e.g.*, muscular dystrophies) is cTn. Based on our experience patients with unexplained increased cTnT with normal cTnI should be also evaluated for possible, clinically still asymptomatic chronic skeletal muscular diseases.

RISK STRATIFICATION BY HS-CTN TESTING-IS THERE ADDITIONAL VALUE COMPARED WITH HIGH-SENSITIVITY C-REACTIVE PROTEIN OR NATRIURETIC PEPTIDE TESTING?

There are no studies to date evaluating hs-CRP or natriuretic peptides together with hs-cTn assays for risk stratification in non-ST-segment elevation myocardial infarctions. Patients in the community who have elevated values of hs-cTn have underlying cardiovascular disease and thus are in the long run at increased risk for ischemic events and heart failure, and hs-cTn was also described as an independent risk marker in the general population^[35-37,39,43,46,47]. However, despite robust statistical predictive value, hs-cTn is similar to hs-CRP and natriuretic peptide testing in the sense that when added to traditional risk factors, it only modestly improves risk stratification and reclassification. There are still insufficient data to as-

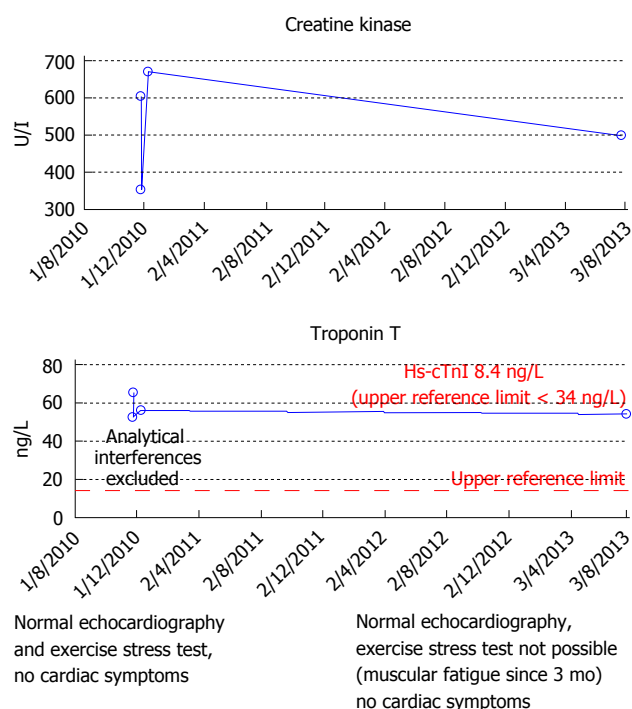


Figure 3 Creatine kinase and high-sensitivity cardiac troponin T and I in a 72-year-old male with late onset limb-girdle muscular dystrophy. This patient presented first to our outpatient clinic in 2010 because of clinically unexplained increased high-sensitivity cardiac troponin (hs-cTnT) concentrations. The echocardiography, exercise stress test and renal function were completely normal, the patient was free of any cardiac symptoms. In 2010 analytical interferences with the hs-cTnT assay were excluded by serial dilution experiments and an interference by heterophilic antibodies could be ruled out by addition of antibody blocking agents to the sample. There was no evidence for macro creatine kinase as well. As the patient had no cardiac symptoms or symptoms suggesting skeletal muscle disease no further work-up was done. In 2013 the patient developed typically symptoms of muscular dystrophy and was again seen in our outpatient clinic. The electrocardiogram and echocardiogram remained normal, he still had no cardiac symptoms but an exercise stress test was no longer possible because of skeletal muscle fatigue. At this visit we found a marked discrepancy between hs-cTnT (moderately increased) and hs-cTnI (normal) suggesting a release of hs-cTnT from chronically injured skeletal muscle by previously already described reexpression of cardiac cTnT isoforms. In retrospect, unexplained hs-cTnT increase in this patient was an early sign of late onset muscular dystrophy.

sess which of these biomarkers is best for risk stratification or whether a multimarker panel including hs-cTn is significantly superior to single marker testing.

CONCLUSION

The 99th percentile concentration of the reference population should be used as the cTn URL and as the medical decision limit. In patients with clinically suspected AMI, the LoD of hs-cTn assays is a useful rule out decision limit with a negative predictive value > 99% even on emergency department admission. The diagnosis of acute myocardial damage requires a significant change with serial hs-cTn testing. At low cTn baseline concentrations (\leq 99th percentile URL) the change in serial testing in order to be clinically significant requires to be a marked (> 100%) increase together with an increase above the URL. In case of borderline increased baseline values (> URL

and \leq 3 times URL) only relative changes > 50% should be considered as clinical significant. In the case of markedly elevated baseline values (> 3 times URL), a minimum change > 20% in follow-up testing is required. It may turn out that for some hs-cTn assays absolute hs-cTn concentration changes perform better than relative changes. Additional testing of other early markers of acute myocardial necrosis, such as myoglobin, CKMB isoforms, or H-FABP is no longer needed. Copeptin testing adds very little as well, particularly, if the LoD is used as a ACS rule out limit on emergency department admission for the hs-cTn assays. Blood sampling in patients with suspicion of AMI should be performed on admission and 3 h later at a minimum. Measurements of hs-cTn should be repeated at 6 h after admission in patients of whom the 3 h values are unchanged but in whom the clinical suspicion of AMI is still high. According to the Universal Definition of Myocardial Infarction^[20] in chest pain patients presenting after 6 h subsequent blood sampling (e.g., after 12 h) is also needed to document a troponin rise or fall as a sign for acute myocardial damage. Blood sampling only at a single time point for troponin measurement is not recommended. cTn is a marker of myocardial necrosis but not a specific marker of AMI. AMI should only be diagnosed when there is a rise and/or fall of cTn together with characteristic symptoms, and/or electrocardiogram or imaging evidence of acute myocardial ischemia. Besides myocardial ischemia one should consider also other alternative causes of acute myocardial damage (e.g., acute heart failure, myocarditis, pulmonary embolism) whenever an elevated hs-cTn test result is obtained. Direct myocardial trauma (e.g., ablation therapy for arrhythmias, surgical incisions of the myocardium, myocardial contusion) also lead to troponin leakage from the myocardium. Stable or inconsistently variable troponin elevations without significant dynamic changes are likely markers of chronic structural heart disease, if analytical interferences (which are rare) have been ruled out.

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Molecular phenotypes of human parvovirus B19 in patients with myocarditis

C-Thomas Bock, Anja DÜchting, Friederike Utta, Eva Brunner, Bui Tien Sy, Karin Klingel, Florian Lang, Meinrad Gawaz, Stephan B Felix, Reinhard Kandolf

C-Thomas Bock, Anja DÜchting, Friederike Utta, Eva Brunner, Karin Klingel, Reinhard Kandolf, Department of Molecular Pathology, University Hospital of Tuebingen, 72076 Tuebingen, Germany

C-Thomas Bock, Bui Tien Sy, Robert Koch Institute, 13353 Berlin, Germany

Florian Lang, Department of Physiology, University of Tuebingen, 72070 Tuebingen, Germany

Meinrad Gawaz, Department of Cardiology and Cardiovascular Medicine, University Hospital of Tuebingen, 72076 Tuebingen, Germany

Stephan B Felix, Clinics for Internal Medicine B, Ernst-Moritz-Arndt-University, 17475 Greifswald, Germany

Author contributions: Bock CT, Gawaz M, Felix SB and Kandolf R conceived and designed the research; Bock CT, DÜchting A, Utta F, Brunner E, Sy BT, Klingel K, Lang F and Kandolf R performed the experiments; Bock CT, Klingel K, Lang F, Gawaz M, Felix SB and Kandolf R analyzed the data; Klingel K, Gawaz M, Felix SB and Kandolf R contributed to reagents/materials/analysis tools; Bock CT and Kandolf R contributed to drafting of the manuscript; all authors read and approved the final manuscript.

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Correspondence to: C-Thomas Bock, PhD, Director, Professor, Department of Molecular Pathology, University Hospital of Tuebingen, Liebermeisterstr. 8, 72076 Tuebingen, Germany. bocke@rki.de

Telephone: + 49-30-187542379 Fax: + 49-30-187542617

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Abstract

AIM: To investigate molecular phenotypes of myocardial B19V-infection to determine the role of B19V in myocarditis and dilated cardiomyopathy (DCM).

METHODS: Endomyocardial biopsies (EMBs) from 498 B19V-positive patients with myocarditis and DCM

were analyzed using molecular methods and functional experiments. EMBs were obtained from the University Hospitals of Greifswald and Tuebingen and additionally from 36 German cardiology centers. Control tissues were obtained at autopsy from 34 victims of accidents, crime or suicide. Identification of mononuclear cell infiltrates in EMBs was performed using immunohistological staining. Anti-B19V-IgM and anti-B19V-IgG were analyzed by enzyme-linked immunosorbent assay (ELISA). B19V viral loads were determined using in-house quantitative real-time polymerase chain reaction (PCR). For B19V-genotyping a new B19V-genotype-specific restriction fragment length polymorphism (RFLP)-PCR was established. B19V-genotyping was verified by direct DNA-sequencing and sequences were aligned using BLAST and BioEdit software. B19V P6-promoter and HHV6-U94-transactivator constructs were generated for cell culture experiments. Transfection experiments were conducted using human endothelial cells 1. Luciferase reporter assays were performed to determine B19V-replication activity. Statistical analysis and graphical representation were calculated using SPSS and Prism5 software.

RESULTS: The prevalence of B19V was significantly more likely to be associated with inflammatory cardiomyopathy (iCMP) compared to uninflamed DCM (59.6% vs 35.3%) ($P < 0.0001$). The detection of B19V-mRNA replication intermediates proved that replication of B19V was present. RFLP-PCR assays showed that B19V-genotype 1 (57.4%) and B19V-genotype 2 (36.7%) were the most prevalent viral genotypes. B19V-genotype 2 was observed more frequently in EMBs with iCMP (65.0%) compared to DCM (35%) ($P = 0.049$). Although there was no significant difference in gender-specific B19V-loads, women were more frequently infected with B19V-genotype 2 (44.6%) than men (36.0%) ($P = 0.0448$). Coinfection with B19V and other cardiotropic viruses was found in 19.2% of tissue

samples and was associated with higher B19V viral load compared to B19V-monoinfected tissue ($P = 0.0012$). The most frequent coinfecting virus was human herpes virus 6 (HHV6, 16.5%). B19V-coinfection with HHV6 showed higher B19V-loads compared to B19V-monoinfected EMBs ($P = 0.0033$), suggesting that HHV6 had transactivated B19V. In vitro experiments confirmed a 2.4-fold increased B19V P6-promoter activity by the HHV6 U94-transactivator.

CONCLUSION: The finding of significantly increased B19V loads in patients with histologically proven cardiac inflammation suggests a crucial role of B19V-genotypes and reactivation of B19V-infection by HHV6-coinfection in B19V-associated iCMP. Our findings suggest that B19V-infection of the human heart can be a causative event for the development of an endothelial cell-mediated inflammatory disease and that this is related to both viral load and genotype.

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Key words: Myocarditis; Dilated cardiomyopathy; Parvovirus B19; B19V-genotypes; B19V co-infection

Core tip: Human parvovirus B19 (B19V) has recently been shown to be an emerging pathogen for inflammatory cardiomyopathy (iCMP). We showed that B19V replication intermediates could be detected in acute and ongoing myocarditis. B19V-genotypes 1 and 2 were predominant although B19V-genotype 2 was more prevalent in iCMP. Further analyses revealed that B19V-coinfection with other cardiotropic viruses does occur, most frequently with human herpes virus 6 (HHV6). In vitro experiments showed that the HHV6 U94-transactivator element could transactivate the B19V-P6-promoter. We suggest that long-term persistence of B19V DNA in the human heart occurs and that active/reactivated B19V-replication can be associated with iCMP in a viral load and genotype-dependent manner.

Bock CT, Dückting A, Utta F, Brunner E, Sy BT, Klingel K, Lang F, Gawaz M, Felix SB, Kandolf R. Molecular phenotypes of human parvovirus B19 in patients with myocarditis. *World J Cardiol* 2014; 6(4): 183-195 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i4/183.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i4.183>

INTRODUCTION

It has been shown that parvovirus B19 (B19V) infection of the myocardium can cause potentially lethal acute myocarditis in infants and adults^[1-3]. Acute B19V-infection of endothelial cells is accompanied by the intravascular accumulation, adhesion and penetration of

inflammatory cells in to vessel walls, leading to an impairment of the myocardial micro-circulation with secondary myocyte necrosis that can mimic myocardial infarction^[1,2]. B19V has been found in high copy numbers in myocardial endothelial cells of small vessels but not in myocytes^[1]. We recently reported that B19V-loads greater than 500 GE/ μ g of isolated nucleic acid identified in endomyocardial biopsies (EMBs) argue for virus-induced myocarditis. In contrast, a low viral load detected in uninflamed hearts has been associated with a latent-type of B19V-infection^[4]. As expected, high viral loads of approximately 3×10^5 GE/ μ g were detected in acute myocardial B19V-infection, while approximately 700 GE/ μ g were found to be characteristically associated with chronic myocarditis^[4]. Notably, a growing number of reports suggests an association between B19V-infection and the development of chronic myocarditis, as well as isolated endothelial and/or diastolic dysfunction^[5-8]. However, the frequent detection of B19V genomes in EMBs of patients clinically suspected of having myocarditis and dilated cardiomyopathy (DCM) and the potential pathogenic role of B19V remains controversial and warrants studies to differentiate viral pathogenic effects from harmless latent B19V-infection^[9,10].

Infection with human parvovirus B19 (B19V) is common, with approximately 70% to 90% of adolescents having anti-B19V IgG detectable in serum^[11]. B19V-infection is usually benign and in children it most commonly manifests with erythema infectiosum (fifth disease)^[12]. The genome of B19V consists of a single stranded DNA molecule of approximately 5.5 kb that contains three major open reading frames coding for the two capsid proteins VP1 and VP2 and the nonstructural protein NS1. Genome diversity divides the genus erythrovirus of the parvoviridae family into three pathogenic human genotypes: PVBAu (genotype 1), Lali-like (genotype 2) and V9-like (genotype 3) viruses^[13]. The three erythrovirus genotypes show different geographical and temporal distributions. Whereas B19V-genotype 1 and 2 can be detected in most populations, genotype 3 seems to be prevalent only in Ghana, France and Brazil^[13-15]. Interestingly, the age distribution of B19V-genotype 1 and 2 infections is different, with B19V-genotype 1 occurring most frequently in individuals born after 1955 while B19V-genotype 2 is predominantly found in individuals older than 50 years^[16,17].

Recent reports have indicated that coinfection with different cardiotropic viruses of the human heart is common^[7,8,18,19]. Human herpes virus 6 (HHV6) has been identified as an important coinfecting pathogen with B19V of the myocardium and resulting in fatal myocarditis in infants^[1,20]. It has been reported that HHV6 is able to transactivate human immunodeficiency virus (HIV) and human cytomegalovirus (HCMV)^[19,21].

In the present study, we explored molecular phenotypes of myocardial B19V-infection in association with patient age, gender, B19V replicative mRNA intermediates, B19V genotype and B19V-coinfection to gain fur-

ther insight into the pathogenesis of B19V-myocarditis as an endothelial-cell mediated inflammatory disease.

MATERIALS AND METHODS

Ethical approval

The study was approved by the ethics committee of the University Hospital of Tuebingen (297/2005). All patients gave written informed consent for EMB analysis to investigate a possible etiology for their disease.

Study population

This cardiopathological clinical and experimental study was designed as a retrospective evaluation of B19V-positive EMBs of 498 consecutive patients (341 male, 157 female, mean age 46.9 ± 15.85 years, ejection fraction $< 45\%$, 2-3 biopsies/patient) with histologically proven myocarditis and DCM who were diagnosed at our institution between 2003 and 2010 (Table 1). In addition to the University Hospitals of Greifswald and Tuebingen, EMBs were obtained from 36 German cardiology centers. Control tissues were obtained at autopsy from 34 victims of accidents, crime or suicide (median age 29 years, kindly provided by Professor Dr. Wehner, Institute of Forensic Medicine, University of Tuebingen). In addition, myocardial tissue obtained from 57 unselected consecutive autopsies at our institute from patients (median age 67.2 years) dying of cardiovascular, cardiopulmonary or tumor-related diseases served as control tissue samples after exclusion of myocarditis and DCM.

Immunohistochemistry and serological testing

Immunohistological staining of paraffin-embedded tissue sections was performed using an avidin-biotin-immunoperoxidase method according to the manufacturer's protocol (Vectastain Elite ABC Kit, Vector, Burlingame, California). The following monoclonal antibodies were used for identification of mononuclear cell infiltrates: CD3 for T cells (Novocastra Laboratories, Newcastle on Tyne, United Kingdom), PGM1 (CD68) for macrophages and natural killer cells, and HLA-DR-Antigen, alpha chain (DAKO, Hamburg, Germany) to assess HLA class II expression on professional antigen-presenting immune cells. According to the World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies, EMBs were considered to have significant inflammation if immunohistochemical staining revealed the presence of focal or diffuse mononuclear infiltrates with > 14 leukocytes per mm^2 (CD3^+ T lymphocytes and/or CD68^+ macrophages) in the myocardium, in addition to enhanced expression of HLA class II molecules^[1].

Anti-B19V-IgM and anti-B19V-IgG (VP1/VP2) were analyzed by enzyme-linked immunosorbent assay (ELISA) (Parvovirus B19-IgM; B19-IgG, DxSelect™ FocusDiagnostics, Germany) according to the manufacturer's instructions.

Table 1 Baseline characteristics of the study population *n* (%)

Characteristic	Value ¹	<i>n</i> ³
Age, yr	46.9 \pm 15.8 ²	498
Male	341 (68.5)	498
Female	157 (31.5)	498
Molecular findings		
B19V-genotype 1	286 (57.4)	498
B19V-genotype 2	183 (36.7)	498
Endomyocardial biopsy results ⁴		
Acute myocarditis	25 (5.0) ($3.2 \times 105 \text{ GE}/\mu\text{g}$) ⁵	498
Inflammatory cardiomyopathy	297 (59.6) ($709 \text{ GE}/\mu\text{g}$) ⁵	498
Dilated cardiomyopathy	176 (35.3) ($392 \text{ GE}/\mu\text{g}$) ⁵	498
Uninflamed control hearts		
B19V-detection	7 (7.7) ($84 \text{ GE}/\mu\text{g}$) ⁵	91
Age (at death), yr	48.1 \pm 20.82	91
Male	49 (53.9)	91
Female	42 (46.1)	91

¹Values are number, absolute and relative frequency of patients; ²Values are expressed as mean and \pm SD; ³Total number of patients; ⁴Histopathology according to the Dallas criteria^[41] supplemented by immunohistochemistry for detection of CD3-positive T-lymphocytes, CD68-positive macrophages and natural killer cells, and HLA class II expression in professional antigen-presenting immune cells as described^[18]; ⁵Values are expressed as mean of B19V-genome equivalents per microgram isolated nucleic acids^[41].

Nucleic acids extraction from EMBs and polymerase chain reaction amplification of viral genomes

Nucleic acids from RNAlater (Qiagen, Hilden, Germany) fixed EMBs and of controls from formalin fixed tissue were extracted as described previously^[11,6]. Polymerase chain reaction (PCR) and reverse transcriptase (RT)-PCR was performed to detect parvovirus B19 (B19V), enteroviruses (EV) (including coxsackieviruses and echoviruses), adenoviruses (ADV), HCMV, Epstein-Barr virus (EBV), and human herpesvirus 6 (HHV6) as previously described^[1]. B19V mRNA was detected using nucleic acids isolated from EMBs. After extensive RNase-free-DNase digestion (20 U; Qiagen, Hilden, Germany) of 30 μL nucleic acid solution for 2 h at 37 °C, the DNase was inactivated for 15 min at 75 °C. 5 μL of the DNase-digested samples were analyzed for removal of B19V DNA by B19V-specific PCR using primer pairs PVB3 and PVB4 and nested PCR primer pairs PVB1 and PVB2 as previously described (Table 2)^[1]. RT-PCR for the detection of B19V-RNA was performed using a one-step RT-PCR reaction kit (Qiagen, Hilden, Germany) and the following primer pairs: first/RT-PCR NS-25 and NS-30 and nested PCR NS-27 and NS-32 (Table 2). RT-PCR reaction was done at 50 °C for 30 min followed by 95 °C for 15 min. PCR was for 35 cycles at 94 °C for 30 s, 53 °C for 30 s, and 72 °C for 45 s, followed by a final extension for 5 min at 72 °C. Nested PCR was performed with an initial denaturation step at 95 °C for 2 min followed by 29 cycles at 95 °C for 30 s, 53 °C for 30 s, and 72 °C for 45 s, followed by a final extension for 5 min at 72 °C. Five μL of each reaction was analyzed using RNase-free agarose gel electrophoresis. Sample processing (DNA/RNA-extraction, template preparation, master-mix preparation)

Table 2 Primer sequences

No	Primer name	Sequences (5' to 3')	Position (numbering according M13178)	1 st , 2 nd (RT/RFLP)-PCR
1	PVB1	GCTAACTCTGTAACCTGTAC	3221-3240	Sense B19V-VP2-PCR (2 nd PCR)
2	PVB2	AAATATCTCCATGGGGTTGAG	3373-3393	As B19V-VP2-PCR (2 nd PCR)
3	PVB3	AGCATGTGGAGTGAGGGGCG	3191-3210	Sense B19V-VP2-PCR (1 st PCR)
4	PVB4	AAAGCATCAGGAGCTATACTTCC	3458-3480	As B19V-VP2-PCR (1 st PCR)
5	NS-25	AAATGCGTGGAAAGTGTAGCT	1628-1647	Sense B19V-NS1-PCR (RT/1 st PCR)
6	NS-27	ATGCGTGGAAAGTGTAGCTGT	1630-1649	As B19V-NS1-PCR (2 nd PCR)
7	NS-30	CCAACTAACAGTTCACGAAAC	2172-2192	Sense B19V-NS1-PCR (RT/1 st PCR)
8	NS-32	TAACAGTTCACGAACTGGTC	2168-2187	As B19V-NS1-PCR (2 nd PCR)
9	NS-38	ATTCCACAAATGCTGATACAC	2498-2519	As RFLP-PCR (1 st PCR)
10	NS-40	AATTGCTGATACACAGCTTTAG	2490-2511	As RFLP-PCR (2 nd PCR)
11	G2170	CAGTTTCGTGAACCTGTTAGT	2170-2189	Sense RFLP-PCR (1 st PCR)
12	G2176	CGTGAACCTGTTAGTGGGGTTGA	2176-2198	Sense RFLP-PCR (2 nd PCR)

As: Antisense; RFLP: Restriction fragment length polymorphism; RT-PCR: Reverse transcriptase-polymerase chain reaction.

and PCR were done in separate laboratory rooms, which are all certified for molecular diagnostics using standard precautions to prevent assay contamination.

Quantitative real-time PCR

B19V viral load was determined using quantitative real-time PCR (qPCR) and calculated according to genome equivalents per microgram isolated myocardial nucleic acid (GE/ μ g) as described previously^[1,4,6]. Dilutions of B19V plasmid DNA and the World Health Organization international B19V DNA standard (code 99/800) were included to standardize the assay. A qPCR of the adenosine triphosphate synthase-6 gene was performed as a control for the addition of equivalent amounts of human DNA as described previously^[6]. All samples were analyzed in duplicate.

DNA sequence analysis and B19V genotype analysis

DNA fragments spanning the B19V NS1/VP1/VP2 coding region (nt 602 to 5014; 4413 nt; numbering according to GenBank accession no. AF162273) were amplified by PCR using primer pairs as described previously^[22]. DNA sequencing was performed in duplicate with purified PCR products in 2 mL BigDye Terminator cycle sequencing mix (Perkin Elmer) and 15 pmol of forward and reverse primers as described previously^[22]. B19V sequences were aligned using BLAST (National Center for Biotechnology Information; <http://www.ncbi.nlm.nih.gov/blast/blast.cgi>). The reliability of alignment was checked using the BioEdit program (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Prototype B19V sequences from GenBank were used as reference sequences (GenBank accession numbers: genotype 1: AB030694, AF113323, AF162273, M13178, DQ225148, DQ225149, DQ225150 and DQ225151; genotype 2: AY064476, AY044266, AY661663 and AY661664; genotype 3: AX003421 and AY083234).

Restriction fragment length polymorphism-PCR for B19V genotyping

In order to determine B19V genotypes, a restriction frag-

ment length polymorphism PCR (RFLP-PCR) was developed using nested PCR. The following primer pairs were used for the first PCR, G2170F and NS-38, and for the second PCR, G2176F and NS-40 (Table 2, Figure 1). Reactions were initially denatured at 94 °C for 4 min followed by 35 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s, followed by a final extension for 10 min at 72 °C. Nested PCR was performed using the primer pair G2176F and NS-40 (Table 2). DNA amplicons (343 bp) were digested with the restriction enzymes HpaI and TaqI in separate reactions (New England Biolabs, Frankfurt, Germany) for 30 min at 37 °C and analyzed by agarose gel electrophoresis. The restriction recognition site for HpaI is only present in B19V-genotype 2 and B19V-genotype 3, resulting in fragment sizes of 117 and 226 bp, respectively, while the TaqI restriction site is only present in B19V-genotype 1 and B19V-genotype 2 (resulting in fragment sizes of 158 and 185 bp) (Figure 1).

Luciferase reporter gene assay

After transfection of human endothelial cells 1 cells with luciferase plasmid constructs containing the B19V P6-promoter region to a final mass of 5 μ g per plasmid containing 0.2 μ g of the β -galactosidase reporter pCMVb-Gal as an internal standard, cells were cultured for 48 h. Cells were co-transfected with an HHV6 U94 expressing vector construct (kindly provided by Dr. S. Aberle, University of Tuebingen, Germany). To measure luciferase, activity cells were harvested, lysed and measured as described previously^[23].

Statistical analysis

Statistical analysis and graphical representation were performed by two-tailed *T*-test and non-parametric Mann-Whitney *U*-test using SPSS statistical software version 16.0 and GraphPad Prism, version 5 (GraphPad Software Inc, San Diego, United States). One-way ANOVA followed by post hoc testing and Tukey's multiple comparison test were also performed. The results were expressed as mean \pm SD. Values below significance level of 0.05 were considered statistically significant.

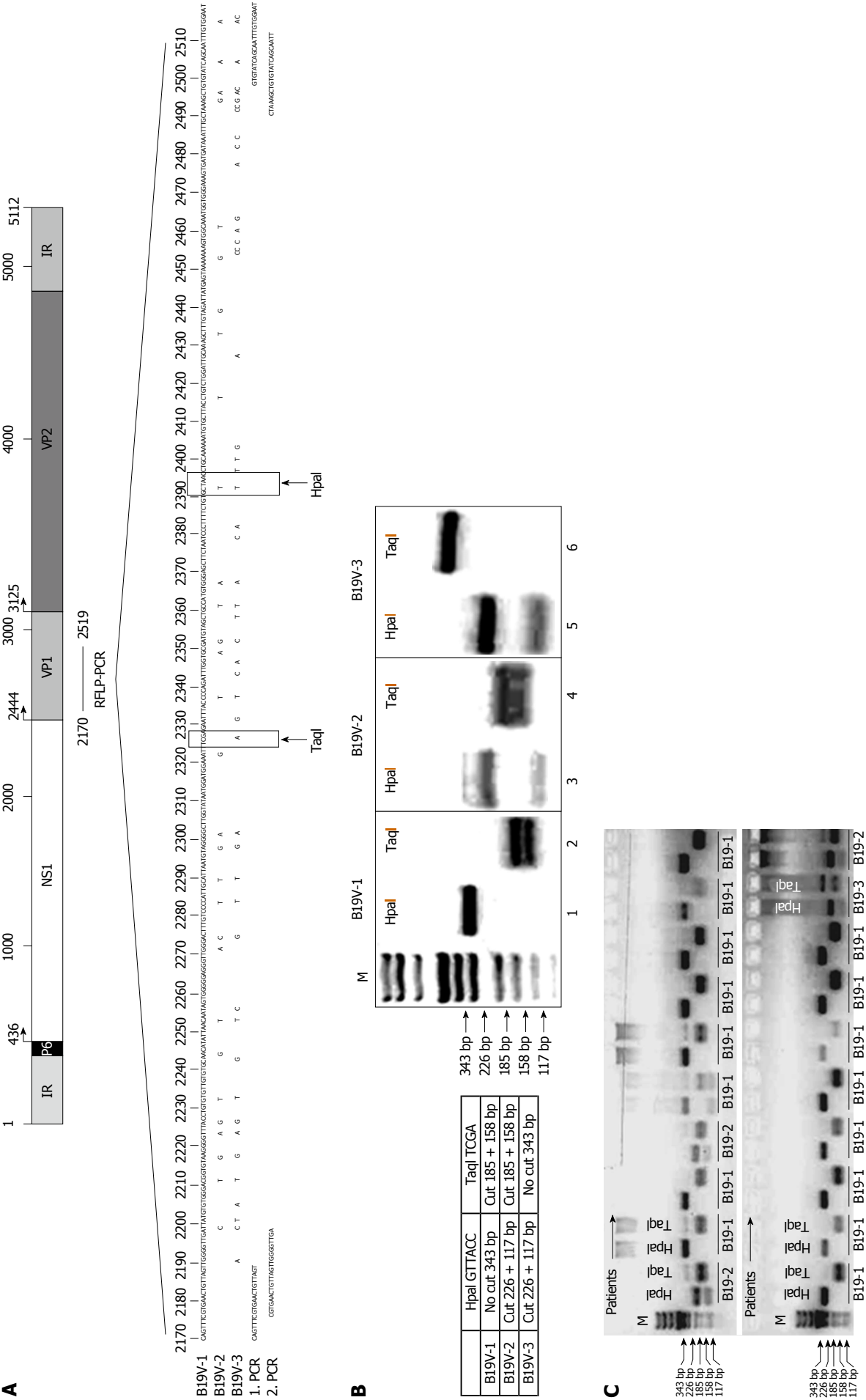


Figure 1 Generation of B19V-genotype 1 to 3 specific restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) in the B19V NS1-VP1u region. Sequences of B19V-1, B19V-2 and B19V-3 showing the RFLP-PCR fragment and HpaI and TaqI restriction enzyme sites (lower panel). Primer positions for 1st and 2nd RFLP-PCR are indicated (1. PCR and 2. PCR, see also Table 2). B: Expected fragment size and digestion pattern after HpaI and TaqI digestions (right panel). Agarose gel electrophoresis showing respective PCR-fragments after HpaI and TaqI digestion for each B19V-genotype (left panel); C: Representative agarose gel electrophoresis of patient-specific B19V RFLP-PCRs. B19V-1: B19V-genotype 1.

RESULTS

Study population

EMBs of 498 B19V-positive patients described in this retrospective study were obtained from 38 clinical centers in Germany between 2003 and 2010 for cardiopathological diagnosis of myocarditis and DCM. In addition, 91 uninflamed hearts without cardiac failure served as a control group. The baseline data of the patients, molecular and cardiopathological findings of the EMBs are provided in Table 1. Quantitative assessment of B19V loads has been described previously, with viral loads of more than 500 GE/ μ g in EMBs as a clinically relevant threshold for the maintenance of myocardial inflammation^[4]. Patients were relatively young (mean age 46.9 ± 15.9 years) and approximately two thirds were men (68.5%) (Table 1). With regards to clinical history, the majority of patients had cardiac symptoms for longer than six months, consistent with chronic heart disease.

Figure 2 illustrates typical histological and immunohistological findings in acute and chronic myocarditis, as well as in uninflamed DCM in B19V-positive human hearts. As expected, acute B19V-myocarditis had a low prevalence of 5% in our study (Figure 2A and 2B, Table 1), while the majority of patients (59.6%) had chronic B19V-myocarditis (Figure 2C and 2D, Table 1). By contrast, chronic DCM without inflammation (Figure 2E and 2F) exhibiting a latent type of B19V-infection was found in 35.3% of our study population (Table 1). Only 7% of 91 uninflamed control hearts were found to harbor latent B19V genomes (Figure 2G and 2H, Table 1).

Replication activity of B19V in myocarditis

In order to determine replication activity of B19V in infected endothelial cells of the myocardium, we determined B19V RNA replication intermediates of 114 randomly selected patients of our cohorts using RT-PCR to amplify the NS1 and VP1 regions of the B19V genome (2 acute, 54 chronic myocarditis, 51 DCM and 7 control hearts). As expected, B19V replicative RNA intermediates could be confirmed in acute B19V-myocarditis as described^[4] (2/2), but also in EMBs of patients with chronic myocarditis harboring viral loads greater than 500 GE/ μ g (16/51) (Figure 3). In contrast, B19V-mRNA intermediates were not observed in EMBs of DCM-patients with uninflamed hearts and viral loads < 100 GE/ μ g, indicating a latent-type of B19V-infection. In addition, B19V-mRNA intermediates were found to be absent in latent B19V-infected normal hearts without inflammation.

B19V-specific IgG and IgM antibodies were detected in the serum by ELISA almost exclusively in B19V-DNA positive EMBs of patients with acute myocarditis (11.4%) but not with DCM or in controls. Overall, B19V-IgG antibodies, but not IgM, were detected in the serum of 91% of patients with chronic myocarditis and in 60.9% with DCM.

Detection of different B19V genotypes in EMBs

Recent reports have shown an association between B19V-

infection of myocardial endothelial cells and the development of viral myocarditis^[1,4,24-26]. However, it is not clear whether B19V-genotypes modulate the outcome of the disease.

In order to determine the B19V genotype confirmed in B19V-positive EMBs, we developed a new RFLP-PCR method spanning the NS1/VP1u region (nt 2170 to 2519; Figure 1A). As shown in Figure 1B, the RFLP-PCR method is capable of discriminating between the known B19V genotypes 1 to 3 by using Hpa1 and Taq1 restriction enzyme digestions. RFLP-PCR patterns of representative patient-specific samples are shown in Figure 1C. In addition, direct sequencing and phylogenetic analysis was performed to verify the specificity of the results by RFLP-PCR. B19V-genotype 1 (286/498; 57.4%) and B19V-genotype 2 (183/498; 36.7%) were the most frequently detected genotypes in persistently infected patients, whereas twenty-five patients with acute B19V-infection showed B19V-genotype 1 (5.0%) (Table 1). In contrast, B19V-genotype 3 infection of the human heart was found to be rare in Germany and only detectable in 0.8% (4/498) of our patients.

B19V genotype-dependent correlation with inflammatory cardiomyopathy

The prevalence of B19V-genotype 1 and B19V-genotype 2 in EMBs was investigated in order to establish if a correlation exists between B19V-genotypes and the occurrence of myocarditis and DCM. B19V-genotype 1 was detected in 176/286 of EMBs (61.5%) with iCMP and in 110/286 samples (38.5%) of DCM-patients without myocardial inflammation, respectively (Figure 4A). A strong trend was observed in the more frequent prevalence of B19V-genotype 2 genomes (total 183/498) in EMBs with iCMP (119/183, 65.0%) compared to DCM (64/183, 35%; $P = 0.048$) (Figure 4A). We found comparable viral loads in B19V-positive EMBs for both B19V-genotype 1 (584 ± 695 GE/ μ g) and B19V-genotype 2 (613 ± 715 GE/ μ g) ($P = \text{ns}$; Figure 4B).

Determination of B19V-genotype specific viral loads in EMBs of patients with iCMP and DCM showed that B19V-genotype 1 load was significantly higher in iCMP samples (706 ± 821 GE/ μ g) compared to DCM (389 ± 343 GE/ μ g; $P < 0.0002$). In keeping with this finding, significantly higher viral loads were observed in B19V-genotype 2 positive EMBs with iCMP (723 ± 846 GE/ μ g) compared to DCM samples (408 ± 270 GE/ μ g; $P = 0.0039$) (Figure 4C).

Age-dependent distribution of B19V-genotype 1 and B19V-genotype 2

Recent publications have shown an association between age and B19V genotype prevalence^[16]. B19V-genotype 2 is considered to be an “older” B19V-variant which is more often found in tissues of patients over the age of 60 years, whereas B19V-genotype 1 is more frequently observed in younger people. In accordance with recent reports, we determined a comparable age-dependent distribution of B19V-genotypes in human hearts^[16,17]. B19V-

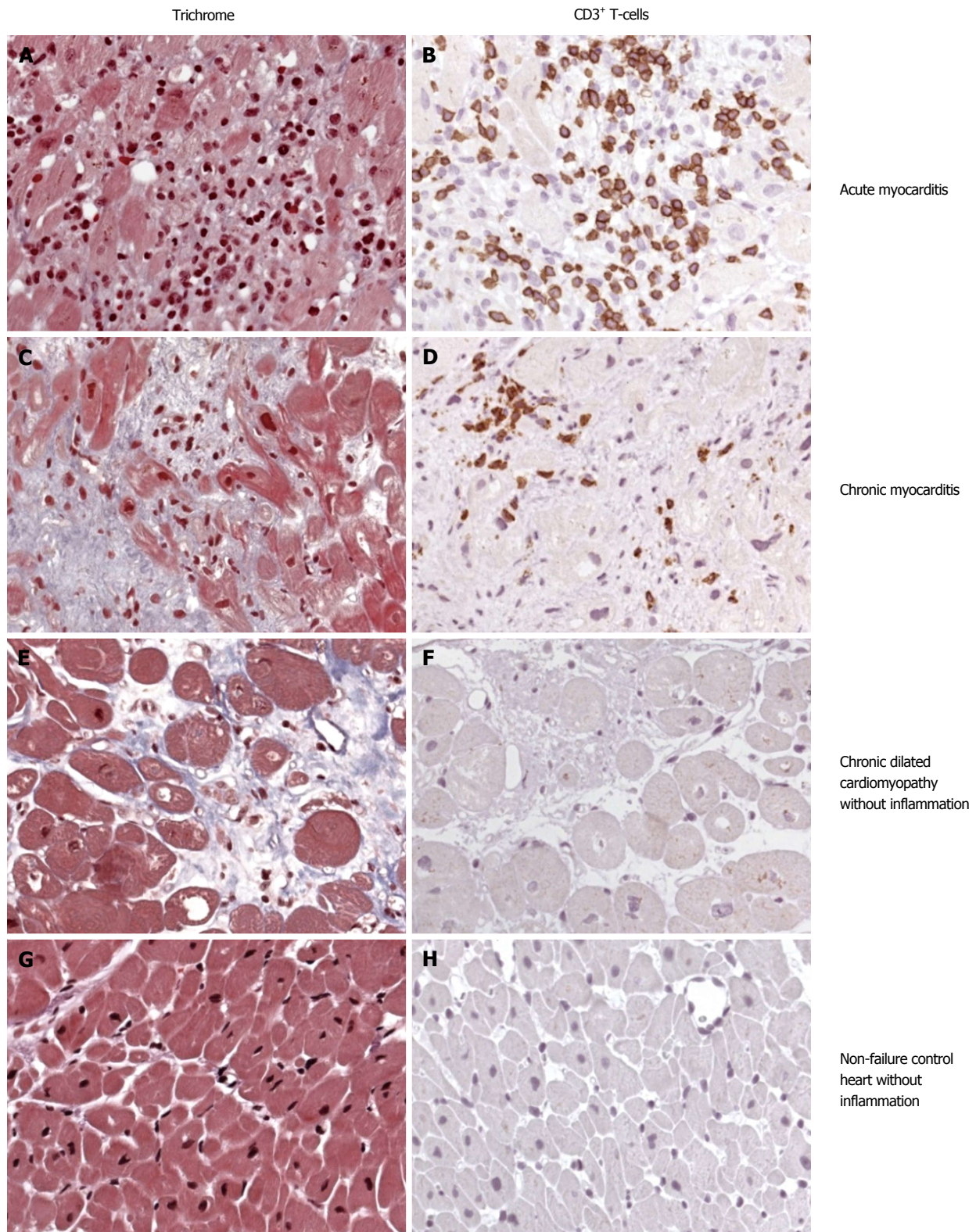


Figure 2 Typical histopathological and immunohistological findings in acute myocarditis (A and B), chronic myocarditis/inflammatory myocarditis (C and D), chronic dilated cardiomyopathy without inflammation (E and F), and non-failure control hearts (G and H). Masson trichrome staining (A, C, E and G) and immunohistological detection of CD3⁺ T-lymphocytes (B, D, F and H).

genotype 1 was predominantly detected in individuals born after 1955, with an average of 43 ± 14 years ($P < 0.0001$), while B19V-genotype 2 was predominantly observed in individuals born before 1955, with an average age of 61 ± 12 years (Figure 5A).

Gender-dependent distribution of B19V-genotype 1 and B19V-genotype 2

We next explored the role of gender in B19V-associated myocarditis. 341/498 (68.5%) EMBs were obtained from male and 157/498 (31.5%) from female patients. There

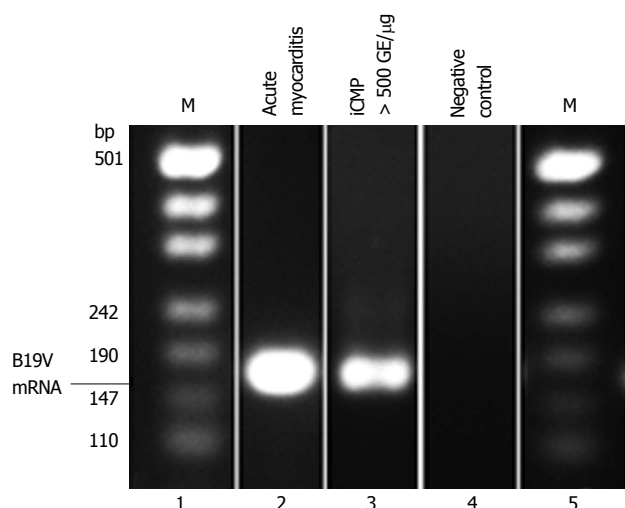


Figure 3 Representative B19V-specific reverse transcription-polymerase chain reaction showing B19V mRNA replication intermediates isolated from endomyocardial biopsies of patients with acute myocarditis (lane 2) and chronic myocarditis/iCMP (lane 3). iCMP: Inflammatory cardiomyopathy.

was no significant difference in age between women (49 ± 16 years) and men (46 ± 16 years; $P = \text{ns}$) (Figure 5B). Furthermore, there was no significant difference in the occurrence of iCMP or DCM in B19V-positive male and female patients ($P = \text{ns}$). A comparison of the B19V-loads in EMBs from men and women showed no significant differences (men 586 ± 679 GE/ μg *vs* women 602 ± 711 GE/ μg) ($P = \text{ns}$). However, there was a trend towards women being more frequently infected with B19V-genotype 2 (66/148, 44.6%) compared to men (117/325; 36.0%; $P = 0.0448$), whereas this was not the case for B19V-genotype 1 infection of men (198/325; 60.9%) and women (81/148; 54.7%; $P = \text{ns}$) (Figure 5C).

Coinfection with cardiotropic viruses detected in B19V-positive EMBs

Recent reports have shown that coinfection of B19V with other cardiotropic viruses, such as HHV6, HCMV, EBV and EV, is not infrequent in viral myocarditis^[18,27]. However, the impact of coinfection on B19V replication has not been determined. Hence, the frequency of B19V-coinfections in EMBs of 473/498 B19V-positive patients with iCMP and DCM was analyzed excluding the twenty-five acute cases of B19V-myocarditis (Table 1). 382/473 (80.8%) EMB samples were infected with B19V alone, while 91/473 (19.2%) were coinfecting with other cardiotropic viruses (Figure 6A). The most prevalent coinfecting virus was HHV6 (78/473, 16.5%). EBV (5/473, 1.1%), EV (4/473, 0.8%) and HCMV (1/473, 0.2%) were detected less frequently (Figure 6B). 3/473 (0.6%) showed evidence of triple-infection with HHV6, EBV and/or HCMV. Only HHV6 subtype B was detected in B19V-positive EMBs. There was no statistically significant difference in the frequency of coinfection with either B19V-genotype 1 or 2 (46.2% *vs* 53.8%; $P = \text{ns}$). Determination of B19V loads in EMBs revealed a statistically significant higher viral load in B19V-coinfection in comparison to

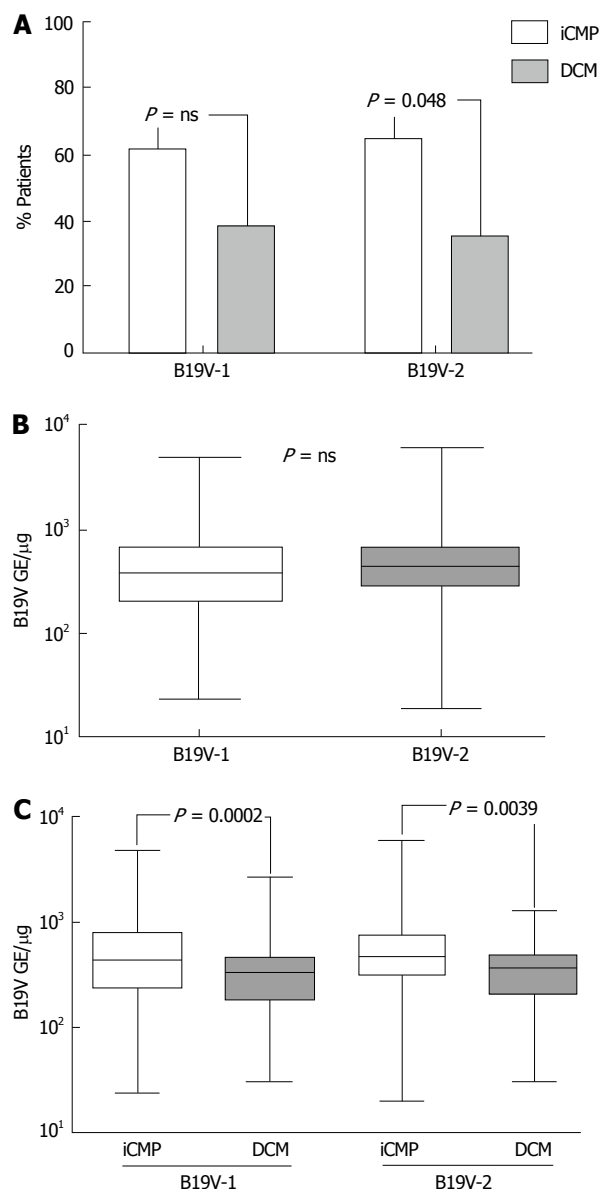


Figure 4 Genotype specific myocardial B19V loads of patients with chronic myocarditis. A: Prevalence of B19V-genotype 1 (B19V-1) and B19V-2 in endomyocardial biopsies of patients with myocarditis [inflammatory cardiomyopathy (iCMP, grey columns) and dilated cardiomyopathy (DCM, white columns). Patient number is given in %; B: qPCR of myocardial of B19V-1 and B19V-2 loads in endomyocardial biopsies (EMB); C: B19V genotype-specific myocardial viral loads in EMBs of patients with chronic myocarditis (iCMP, white columns) and DCM (grey columns) determined by qPCR. One-way Anova was highly significant ($P < 0.0001$). $P < 0.05$ is statistically significant (two-tailed T-test). qPCR: Quantitative real-time polymerase chain reaction.

B19V-monoinfection (754 ± 967 *vs* 552 ± 615 GE/ μg ; $P = 0.0012$) (Figure 6C). Analysis of the prevalence of B19V genotypes showed that B19V-genotype 2 was more frequently associated with B19V coinfection in comparison to B19V-monoinfection (36.7% *vs* 48.3%; $P = 0.023$), whereas this was not the case for B19V-genotype 1 (63.3% *vs* 51.7%; $P = \text{ns}$) (Figure 6D). Interestingly, B19V loads were significantly increased in iCMP samples of B19V-coinfecting in comparison to B19V-monoinfected patients with iCMP (934 ± 1122 *vs* 650 ± 723 GE/ μg ; $P = 0.0157$) (Figure 6E). No difference in B19V loads was found in

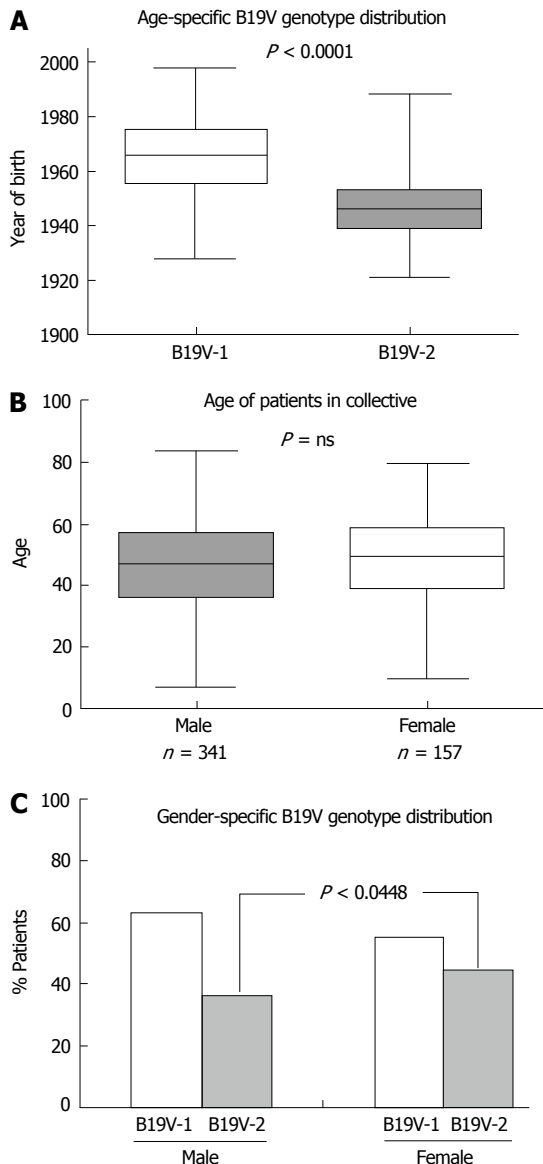


Figure 5 Age and gender dependent distribution of B19V-genotypes in endomyocardial biopsies of patients with myocarditis. A: Distribution of B19V-genotype 1 (B19V-1) and B19V-2 according to year of birth; B: Gender-specific mean age of our patient cohort; C: Gender-specific distribution of B19V-1 (white columns) and B19V-2 (grey columns). $P < 0.05$ is statistically significant (two-tailed T-test).

DCM samples of B19V mono- and coinfecting patients (396 ± 336 vs 371 ± 205 GE/ μ g; $P = ns$) (Figure 6E). Further analysis showed that the increased B19V load was mainly due to coinfection of B19V with HHV6 (869 ± 992 vs 552 ± 615 GE/ μ g; $P = 0.0006$) in comparison to B19V coinfections with EV or EBV (466 ± 254 and 350 ± 218 GE/ μ g, respectively; $P = ns$) (Figure 6F). Functional analysis demonstrated that the HHV6 U94 transactivator, which shows similarities to parvovirus NS1/Rep transactivator^[28], is able to transactivate the P6-promoter of B19V by 2.4 fold using luciferase promoter activity assays (Figure 6G).

DISCUSSION

Parvovirus B19 has been reported to be the causative

agent for a wide variety of clinical conditions, ranging from asymptomatic infection to fifth disease in childhood (erythema infectiosum), hydrops fetalis and transient aplastic anemia^[12,29,30]. In addition to EV, the classical causative pathogens of myocarditis^[8,27], B19V has also been described to account for inflammatory cardiomyopathy (iCMP)^[1,24,25,27]. However, the relatively high prevalence of B19V in EMBs has led to controversy around the role and mechanism of B19V in the pathogenesis of myocarditis^[9,31].

It has been demonstrated that B19V infects endothelial cells of small myocardial blood vessels, which can result in the impairment of myocardial microcirculation^[1,2,24,25], endothelial dysfunction^[6] and secondary necrosis of myocardial cells^[2]. A recent report by Streitz *et al.*^[32] demonstrated the presence of anti-B19V NS1-specific T-cell response in B19V-associated myocarditis, supporting the notion that B19V-infection has a pathogenic role in the development of iCMP. It has been shown that active persistent B19V-infection is responsible for triggering inflammatory response in the myocardium^[18]. Recently, we demonstrated that B19V and the viral proteins of B19V play an important pathophysiological role in modulating inflammatory signaling, regulation of pro-apoptotic processes and modulation of the intracellular Ca^{2+} -activity leading to endothelial dysfunction^[26,33,34].

It is important to note that B19V DNA is more frequently found in EMBs of patients with chronic myocarditis (59.6%) compared to control cardiac tissue samples from individuals without heart failure (7.7%), a finding consistent with other reports^[2,35]. Based on the assessment of B19V-myocarditis and DCM, a viral load threshold of greater than 500 B19V-GE/ μ g has been suggested to be of clinical significance for the maintenance of myocardial inflammation^[4]. This correlates with the finding of B19V RNA replication intermediates predominantly in myocardial tissue of patients with acute and chronic myocarditis, but not in uninflamed hearts (Figure 2). However, the detection of B19V-RNA replication intermediates in EMBs is challenging because of sampling errors and RNA copy numbers that may be below limit of detection.

In order to determine the B19V-genotype distribution in EMBs of patients with iCMP/DCM, we developed a RFLP-PCR technique to discriminate between the three B19V-genotypes (Figure 3). In line with recent publications, we found that B19V-genotype 1 was the most common genotype, followed by B19V-genotype 2, while B19V-3 was rarely found^[17]. This is not surprising as B19V-genotype 3 is most commonly found in Ghana, Brazil and France^[13,14,36].

While B19V was detected more frequently in EMBs of patients with iCMP compared to DCM that lacked inflammatory cell infiltrates, B19V viral loads of both B19V-genotypes 1 and 2 were significantly higher in iCMP samples (approximately 700 GE/ μ g) compared to DCM (approximately 400 GE/ μ g) (Figure 4). These findings indicate that regardless of the B19V-genotype, B19V-iCMP is associated with significantly higher viral

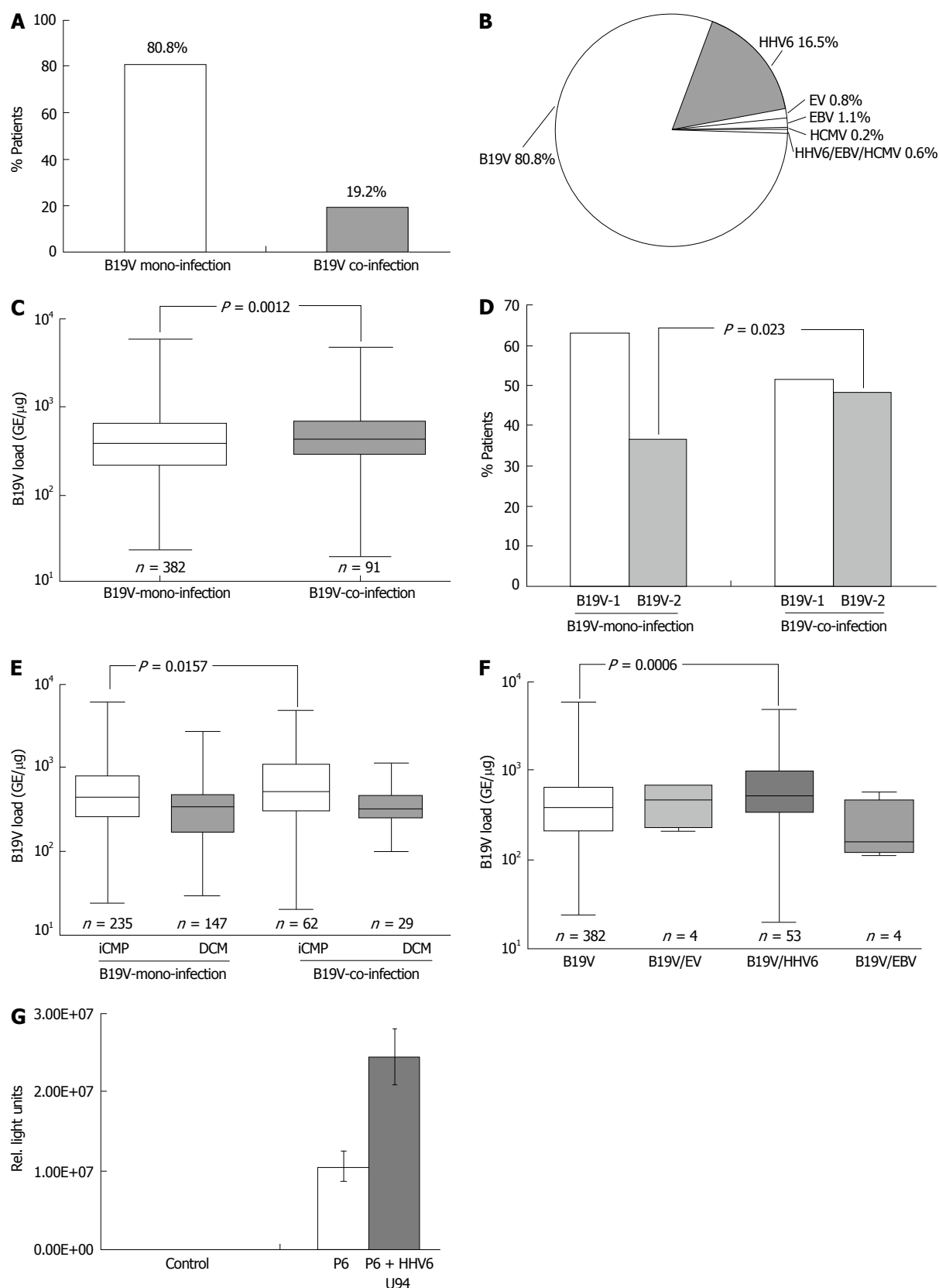


Figure 6 Distribution of B19V-coinfection with cardiotropic viruses. A: In endomyocardial biopsies determined by virus-specific nPCR; B: Frequency of B19V-coinfection with cardiotropic viruses; C: qPCR of B19V loads in B19V mono- and co-infection; D: Distribution of B19V-genotype 1 and 2 in B19V mono- and co-infection in endomyocardial biopsies (EMBs); E: B19V loads of B19V mono- and co-infection with cardiotropic viruses. One-way Anova was highly significant ($P < 0.0001$); F: B19V loads of B19V mono- and co-infection with cardiotropic viruses. One-way Anova was highly significant ($P = 0.0091$); G: Luciferase reporter assay to determine transactivation capacity of the HHV6-U94 transactivator on the B19V P6-promoter activity. $P < 0.05$ is statistically significant (two-tailed T-test). HHV6: Human herpesvirus 6; EV: Enterovirus; HCMV: Human cytomegalovirus; EBV: Epstein-Barr virus; DCM: Dilated cardiomyopathy; iCMP: Inflammatory cardiomyopathy; qPCR: Quantitative real-time polymerase chain reaction.

loads than B19V-DCM.

As with the age-dependent distribution of B19V-genotype 1 and 2 in various tissue samples (*e.g.*, skin, synovium, liver, and heart)^[15-17], we found that B19V-genotype 1 was predominantly detected in younger people (mean age 43 years), while B19V-genotype 2 was mostly observed in patients older than 60 years (Figure 5). This age-associated difference in frequency of genotypes is thought to be due to the reported lifelong persistence of viral genomes in humans^[15,16,29]. Analysis of gender dependency of B19V genotype distribution and viral loads did not show any significant differences. However, a significantly higher proportion of women were found to be persistently infected with B19V-genotype 2 in contrast to men ($P < 0.05$) (Figure 5).

It can be hypothesized that B19V can be reactivated from long-term persistent or latent infection by viral and/or host-specific determinants. B19V-coinfection with other cardiotropic viruses like EV, ADV and HHV6 may contribute to the severity of B19V-myocarditis^[4,7,8,20], possibly by reactivating B19V replication and thereby enhancing virus specific host immune responses, tissue inflammation and the progression to chronic heart failure^[37]. This is reminiscent of the increased replication of HIV caused by coinfection with HHV6 and HHV8^[21,38]. Our finding show that coinfection with B19V and other cardiotropic viruses, particularly HHV6, is not infrequent and is associated with higher B19V loads in EMBs. HHV6 cannot only infect and replicate in endothelial cells^[39], but also encodes a viral transactivator^[37] called U94 which is homologous to the B19V-NS1 transactivator^[28]. By using promoter activity assays, we have shown that the HHV6 U94 transactivator also transactivates the B19V-P6 promoter, resulting in enhanced B19V loads in B19V/HHV6 coinfection (Figure 6).

Our data shows that B19V is capable of long-term persistence in the human heart, even lasting for decades, and that active B19V replication is associated with the development of iCMP. To establish a clinically relevant link between B19V-infection and the development of iCMP, it is important to develop molecular diagnostic techniques to determine myocardial viral loads and sequence analysis to verify the association between myocardial disease and genotype-specific B19V isolates. Notably, persistently low replicating and latent B19V-infection may be reactivated by coinfecting cardiotropic viruses, especially HHV6. Our findings together with other recently published data^[1,6,18,25,26,40] argue an important role for B19V in the development of iCMP.

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COMMENTS

Background

Viral and post viral myocarditis is the major cause of acute and chronic dilated cardiomyopathy (DCM). Human parvovirus B19 has been identified as a new emerging pathogenic agent in the etiology of inflammatory cardiomyopathy (iCMP). However, the role of B19V-infection in the development of chronic myocarditis is still unclear. The authors have recently demonstrated that endothelial cells but not cardiac myocytes are B19V-specific target cells in patients with B19V-associated myocarditis. Furthermore, B19V was not detected frequently in patients with unexplained isolated diastolic dysfunction. Molecular phenotypes such as patient age, gender, B19V replicative mRNA intermediates, B19V genotype and B19V-coinfection of myocardial B19V-infection contributing to the etiopathogenesis of B19V-myocarditis as an endothelial-cell mediated inflammatory disease have not been described so far.

Research frontiers

The identification of various viral nucleic acids by polymerase chain reaction in the myocardium of patients with suspected myocarditis led to the hypothesis that acute and chronic myocarditis may develop as a result of infection, not only with enteroviruses (Coxsackievirus B3), but also with other cardiotropic viruses, such as parvovirus B19. In this regard, the research hotspot is how molecular phenotypes can contribute to the pathogenic role of myocardial B19V-infection in myocarditis and DCM.

Innovations and breakthroughs

The study results showed that B19V RNA replication intermediates could be determined mainly in acute and ongoing myocarditis, indicating active replication of B19V. B19V-genotypes 1 and 2 were predominant with a more frequent prevalence of B19V-genotype 2 in iCMP. Furthermore, B19V-coinfections with other cardiotropic viruses can occur, most frequently with human herpes virus 6 (HHV6). Functional experiments showed that the HHV6 U94-transactivator could transactivate the B19V-P6-promoter. From these findings it is suggested that long-term persistence of B19V DNA in the human heart occurs and that active/reactivated B19V-replication can be associated with iCMP in a viral load and genotype-dependent manner.

Applications

To establish a clinically relevant link between B19V-infection and the development of iCMP, it is important to pursue molecular diagnostic techniques to determine myocardial B19V loads and to verify genotype-specific B19V isolates. Notably, persistent low replicating and even latent B19V-infection may be reactivated by coinfecting cardiotropic viruses, especially HHV6.

Terminology

Parvovirus B19 was assumed to be an agent of human disease when its association with erythema infectiosum (fifth disease), hydrops fetalis and transient aplastic anemia was demonstrated in the 1980s. During the last few years, a growing number of reports have been published demonstrating an association between B19V and many other clinical diseases, like arthritis, myocarditis, various vasculitis syndromes, hepatitis and neurological disorders. A growing number of reports have suggested an association between B19V infection with acute and chronic cardiac diseases. Myocarditis is the term used to indicate infectious, toxic or autoimmune processes causing inflammation of the heart and represents a non-ischemic inflammatory heart disease with a highly variable clinical outcome. In most cases this disease is self-limiting; however, it may also lead to acute heart failure, resulting in early death or heart transplantation. Myocarditis can also mimic acute myocardial infarction. ICMP was included as a subtype of the specific cardiomyopathies and defined "as myocarditis in association with cardiac dysfunction" by the World Health Organization.

Peer review

The paper deals with the interesting subject of molecular phenotypes of human parvovirus B19 in patients with myocarditis. Both the specific virus, which is a cause of important pathologies, as well as myocarditis, an entity that can affect great portions of a population, among them young, otherwise healthy individuals, are very interesting subjects with an impact on the general practice of internists, cardiologists, general physicians, pathologists, biologists and genetic scientists. The paper deals with the aforementioned interesting subject in a thorough way.

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Coronary artery disease in congenital single coronary artery in adults: A Dutch case series

Salah AM Said, Willem G de Voogt, Suat Bulut, Jacques Han, Peter Polak, Rogier LG Nijhuis, Jeroen W op den Akker, Andries Slootweg

Salah AM Said, Rogier LG Nijhuis, Andries Slootweg, Department of Cardiology, Hospital Group Twente Almelo-Hengelo, 7555 DL Hengelo, The Netherlands

Willem G de Voogt, Department of Cardiology, St. Lucas-Andreas Hospital, 1061 AE Amsterdam, The Netherlands

Suat Bulut, Department of Cardiology, Gelre Hospital Zutphen, 7200 GZ Zutphen, The Netherlands

Jacques Han, Department of Cardiology, Hospital De Sionsberg, 9101 DC Dokkum, The Netherlands

Peter Polak, Department of Cardiology, St. Anna Hospital, 5664 EH Geldrop, The Netherlands

Jeroen W op den Akker, Department of Radiodiagnostics, Hospital Group Twente Almelo-Hengelo, 7555 DL Hengelo, The Netherlands

Author contributions: All authors contributed equally to the writing of the manuscript.

Correspondence to: Dr. Salah AM Said, Department of Cardiology, Hospital Group Twente Almelo-Hengelo, Geerdinksweg 141, 7555 DL Hengelo, The Netherlands. samsaid@home.nl

Telephone: +31-74-2905286 Fax: +31-74-2905289

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Abstract

AIM: To assess the current diagnostic and therapeutic management and the clinical implications of congenital single coronary artery (SCA) in adults.

METHODS: We identified 15 patients with a SCA detected from four Dutch angiography centers in the period between 2010 and 2013. Symptomatic patients who underwent routine diagnostic coronary angiography (CAG) for suspected coronary artery disease and who incidentally were found to have isolated SCA were analyzed.

RESULTS: Fifteen (7 females) with a mean age of 58.5 ± 13.78 years (range 43-86) had a SCA. Conventional

CAG demonstrated congenital isolated SCA originating as a single ostium from the right sinus of Valsalva in 6 patients and originating from the left in 9 patients. Minimal to moderate coronary atherosclerotic changes were found in 4, and severe stenotic lesions in another 4 patients. Seven patients were free of coronary atherosclerosis. Runs of non-sustained ventricular tachycardia were documented in 2 patients, one of whom demonstrated transmural ischemic changes on presentation. Myocardial perfusion scintigraphic evidence of transmural myocardial ischemia was found in 1 patient due to kinking and squeezing of the SCA with an interarterial course between the aorta and pulmonary artery. Multi-slice computed tomography (MSCT) was helpful to delineate the course of the anomalous artery relative to the aorta and pulmonary artery. Percutaneous coronary intervention was successfully performed in 3 patients. Eight patients were managed medically. Arterial bypass graft was performed in 4 patients with the squeezed SCA.

CONCLUSION: SCA may be associated with transient transmural myocardial ischemia and aborted sudden death in the absence of coronary atherosclerosis. The availability and sophistication of MSCT facilitates the delineation of the course of a SCA. We present a Dutch case series and review of the literature.

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Key words: Congenital heart disease; Coronary artery anomaly; Coronary angiography; Single coronary artery; Coronary artery disease; Multi-slice computed tomography

Core tip: A Dutch case series of 15 adult patients with congenital isolated single coronary artery (SCA) are presented. Conventional coronary angiography demon-

strated congenital isolated SCA originating as a single ostium from the right sinus of Valsalva in 6 patients and originating from the left in 9 patients. SCA may be associated with symptomatic transient transmural myocardial ischemia, non-sustained ventricular tachycardia, and aborted sudden death in the absence or presence of coronary atherosclerosis. The availability of multi-slice computed tomography (MSCT) and cardiovascular magnetic resonance imaging facilitates the delineation of the course of the anomalous vessel. MSCT was helpful to delineate the course of the anomalous artery relative to the aorta and pulmonary artery. Percutaneous coronary intervention was successfully performed in 3 patients. Eight patients were managed medically. Arterial bypass graft was performed in 4 patients with the squeezed SCA. The literature addressing SCA is reviewed.

Said SAM, de Voogt WG, Bulut S, Han J, Polak P, Nijhuis RLG, op den Akker JW, Slootweg A. Coronary artery disease in congenital single coronary artery in adults: A Dutch case series. *World J Cardiol* 2014; 6(4): 196-204 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i4/196.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i4.196>

INTRODUCTION

A single coronary artery (SCA) is defined as a single aortic orifice or origin providing for all of the coronary blood perfusion of the entire myocardium^[1-3]. In 1967, Halperin *et al*^[4] reported the first ante mortem angiographic diagnosis of SCA arising from the left sinus of Valsalva (LSV). SCA is a rare congenital anomaly and occurs as an incidental finding in approximately 0.066% of the coronary angiography (CAG) population^[5]. SCA has been reported in association with and without atherosclerotic changes^[6,7] or in association with coronary artery fistulas^[8,9], bicuspid aortic valves, and with hypertrophic cardiomyopathy^[1,7,10,11].

An equal distribution is found between SCA originating from the right sinus of Valsalva (RSV) and the LSV^[2,12]. Exact delineation of the course of the abnormal coronaries relative to the aorta and pulmonary artery is of major importance as myocardial ischemia during exertion can be caused by kinking or squeezing of the branches of the anomalous SCA between the aorta and pulmonary artery. CAG is the first diagnostic tool in the detection of a SCA. Once abnormal coronary arteries are suspected, multi-slice computed tomography (MSCT) and cardiac magnetic resonance (CMR) imaging^[13] scans are excellent tools for non-invasive determination of the course of the abnormal coronaries relative to the aorta and pulmonary artery^[14]. Determination of the course of incidentally found congenital coronary anomalies during routine CAG without the direct availability of CMR or MSCT scanning is challenging.

We discuss the clinical presentation and angiographic findings of 15 adult symptomatic patients with congenital

isolated SCA incidentally found during routine CAG.

MATERIALS AND METHODS

Between 2010 and 2013, 15 adult patients with a mean age of 58.5 ± 13.78 years (range 43-86) were diagnosed with a SCA during CAG in 4 Dutch angiography centers (Hospital Group Twente, Almelo; St. Lucas Andreas Hospital, Amsterdam; St. Anna Hospital, Geldrop; Hospital Group Twente, Hengelo; and Gerle Hospital, Zutphen). Indications for CAG were angina pectoris, dyspnea, and syncope.

The angiograms were reviewed by at least two experienced cardiologists who reached a consensus on the origin and course of the SCA. The angiographic variations and the course of the anomalous artery were defined according to the classification of Lipton *et al*^[1]. The definition of SCA was adopted from Angelini *et al*^[15] and defined as an isolated coronary artery arising from the sinus of Valsalva through a single ostium and with no evidence of a second ostium, thus being responsible for supplying blood to the entire myocardial tissue, regardless of its distribution.

Significant atherosclerosis was defined as luminal narrowing of $\geq 75\%$ detected in a main branch of the epicardial coronary arteries. Patients were categorized as having significant single, double, or triple vessel disease when a significant lesion was found in one or more coronary artery branches arising from the SCA and supplying the right coronary artery (RCA), circumflex (Cx), or left anterior descending coronary artery regions. A 12-lead ECG was performed in all patients.

An exercise tolerance test (ETT) was performed in 10 patients, myocardial perfusion test [methoxy-isobutylisonitrile (MIBI) scan] in five patients, and ¹³ammonia-adenosine positron emission tomography (positron emission tomography-computed tomography) scan in one patient. MSCT was performed in 6 patients using a retrospective ECG-gated procedure (128-slice, Philips Medical Systems, Best, The Netherlands).

RESULTS

Patients comprised 8 males and 7 females, aged between 43 and 86 years (mean 58.5 ± 13.78). Effort angina pectoris was found in 6 patients, 4 had dyspnea on exertion, 4 complained of atypical chest pain, fainting and pre-syncope, 1 had recurrent syncopal attacks, and 2 presented with acute coronary syndrome. Patients' characteristics are presented in Table 1. Between 2010 and 2013, 8917 coronary angiograms were performed in the 4 Dutch angiography centers all together, with an incidence of 15/8917 (0.017%).

On the resting ECG, all patients were in sinus rhythm and had normal PR intervals. Two patients (patients 3 and 15) had a complete left bundle branch block. One patient (patient 5) had inverted T-waves in the inferior leads. In another patient (patient 8), ECG evidence of an old infero postero lateral infarction was shown. Short

Table 1 Patients' characteristics

Case /gender/age	Clinical presentation	Rest ECG	Risk factors	ETT	MIBI scan	CAD	Management	CAG ¹ classification	MSCT
1/F/45	AP, DOE	SR	-	Inconclusive	NA	None	CMM	R-II P	NA
2/M/56	DOE	SR	+	Positive	NA	Intermediate lesion	CMM	R- I	Overestimation of the Cx-lesion
3/F/60	AP	RD SR LBBB	-	Ischemia IL NA	NA	FFR 0.93 Mild	CMM	R-III	NA
4/M/86	ACS	SR	+	Negative	NA	Significant	PCI	L- I	NA
5/M/63	Effort AP	SR	+	Positive	NA	Significant	PCI	L- II A	NA
6/F/43	ACP, fainting and pre-syncope	Negative T Inferior leads SR	+	Inconclusive	Positive ¹⁵ N-adenosine PET-CT: normal	None	CABG	L- II B	Course: between aorta and pulmonary artery
7/M/48	AP, syncope	SR NSVT (5 beats)	+	Negative	Negative	None Ergonovine test: No spasm	CMM	L- I	NA
8/F/53	DOE, palpitation	SR RD NSVT (20 beats)	+	NA	Positive	Intermediate lesion	CMM	R- II A	NA
9/M/46	AP, palpitation	SR	-	NA	NA	None	CMM	R- II A	NA
10/M/63	AP	SR	+	Positive	NA	Significant	CABG	L- II B	Course: between aorta and pulmonary artery
11/F/83	NSTEMI	SR	+	NA	NA	Significant	PCI	R-III	NA
12/F/47	ACP	SR	+	Negative	NA	None	CMM	L- II A	NA
13/F/53	CP syncope	SR	-	Negative	Negative	None	CABG	L- II B	Course: between aorta and pulmonary artery
14/M/72	DOE	SR	+	NA	NA	Intermediate lesion	CABG	L- II B	Course: between aorta and pulmonary artery
15/M/41	ACP	SR LBBB	+	Negative	Negative	None	CMM	L- II A	Benign course

¹Classification according to Lipton *et al*^[1]. A: Anterior; AP: Angina pectoris; ACP: Atypical chest pain; ACS: Acute coronary syndrome; B: Between aorta and pulmonary artery; CAD: Coronary artery disease; CMM: Conservative medical management; ETT: Exercise tolerance test; F: Female; FFR: Fractional flow reserve; M: Male; CABG: Coronary artery bypass grafting; DOE: Dyspnoea on exertion; N: Normal; NA: Not available; P: Posterior to the aorta; PCI: Percutaneous coronary intervention; R: Right; L: Left; LBBB: Left bundle branch block; MSCT: Multi-slice computed tomography; SR: Sinus rhythm; -: Absent; +: Present (hypertension, smoking, obesity, hypercholesterolemia); PET-CT: Positron emission tomography-computed tomography; NSVT: Non-sustained ventricular tachycardia; RD: Repolarization disturbances; Cx: Circumflex; CAG: Coronary angiography; NSTEMI: Non-ST elevation myocardial infarction; MIBI: Methoxy-isobutyl-isonitrite; CP: Chest pain.

runs of non-sustained ventricular tachycardia (NSVT), varying from 5 to 20 beats/min with a frequency of 176/min and duration of 1800 ms, were documented in 2 patients (patients 7 and 8) (Figure 1).

ETT was inconclusive in 2 patients (1 and 6). Diagnostic CAG showed no significant coronary artery lesions in either patient. In patient number 6, the PET scan was positive due to kinking and squeezing of the SCA with a course between the aorta and pulmonary artery. This patient underwent coronary artery bypass grafting (CABG) whereby a mammary arterial graft was anastomosed to the RCA. In 3 patients (2, 5 and 10), the ETT was positive for myocardial ischemia. Of the 3 patients with positive ETT, 1 had significant CAD and underwent percutaneous coronary intervention (PCI). The other 2 patients demonstrated an intermediate lesion distally located in the coronary arterial tree and were managed medically. The ETT was negative in 5 patients (4, 7, 12, 13 and 15). Despite a negative ETT, patient number 4 showed a significant coronary lesion on CAG and underwent PCI. Pa-

tient number 7 had no significant coronary artery lesions and the ergonovine test was negative. Patient number 8 had a positive MIBI scan and CAG showed an intermediate lesion, which was managed medically. MSCT scan of 5 patients (6, 10, 13, 14 and 15) demonstrated an interarterial course and they underwent CABG, whereby a mammary arterial graft was anastomosed to the RCA in 4 and the fifth showed a benign course.

Conventional CAG demonstrated a SCA originating as a single ostium from the RSV in 6 patients and SCA originating as a single ostium from the LSV in 9 patients (Figures 2 and 3).

MSCT was performed in 6 patients (2, 6, 10, 13, 14 and 15). In patient 2, MSCT confirmed the diagnosis of SCA but gave an overestimation of the severity of the coronary lesion in the Cx trajectory, which was not significant (0.93) by fractional flow reserve measurement (Figure 4). In 4 patients (6, 10, 13 and 14), SCA was also proven by MSCT depicting clearly the course of the SCA running between the aorta and the pulmonary artery

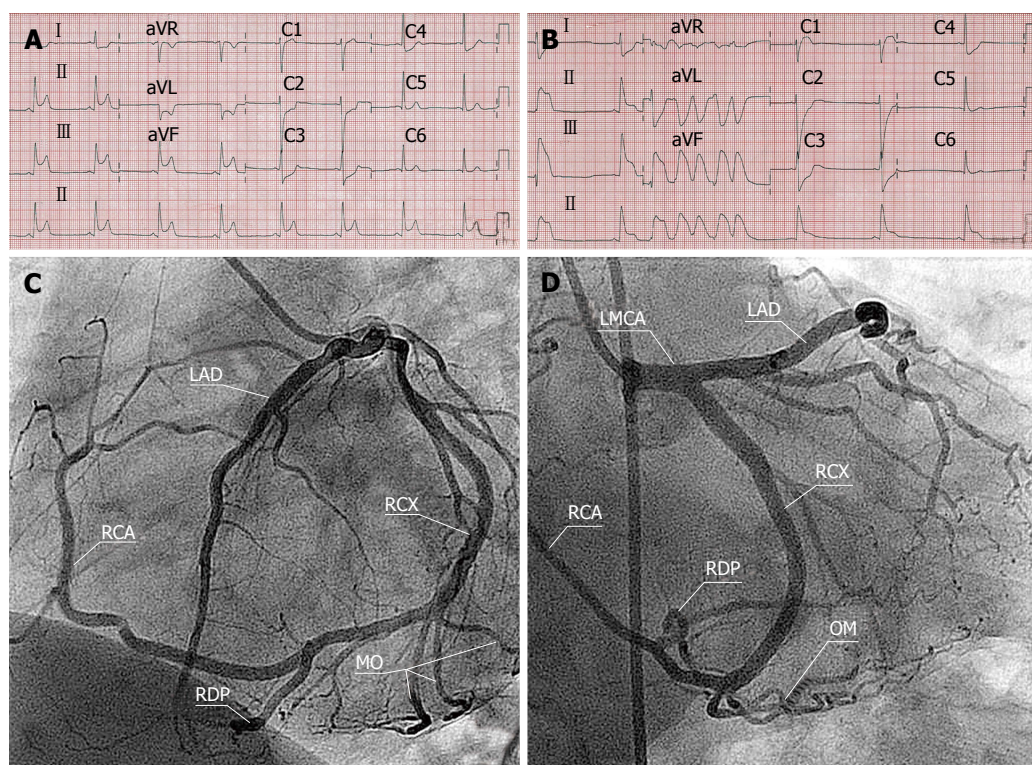


Figure 1 Resting electrocardiograph. A: Electrocardiograph during chest pain depicting transmural ischemia in the infero-posterior leads; B: Followed by a non-sustained monomorphic ventricular tachycardia; C: Coronary angiography showed absence of the right coronary ostium and a single coronary artery arising from the left sinus of Valsalva with normal origin of the left coronary artery (LCA) having normal anatomical course of the left main stem, the left anterior descending, and the circumflex artery (Lipton L- I); D: The LCA supplies the entire myocardial tissue. No significant stenoses were found. The right coronary artery (RCA) appeared as a continuation of the distal left circumflex artery to the right atrioventricular groove and terminated near the RSV (Lipton L- I). LAD: Left anterior descending; LMCA: Left main coronary artery; RCX: Ramus circumflexus; RDP: Ramus descending posterior; OM: Obtuse marginal.

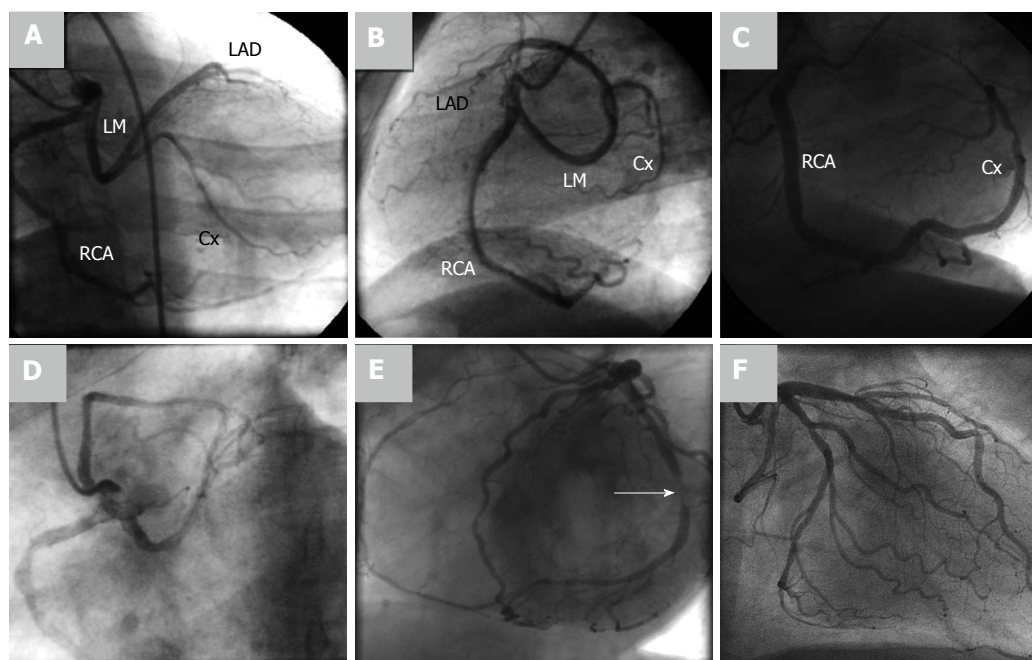


Figure 2 Coronary angiography frame. A: Coronary angiography frame of right anterior oblique projection with cranial angulation; B: Left lateral (LL) projection showing a single origin of the right and left coronary arteries from a common right coronary ostium (Lipton R- II P), the long curved left main stem and right dominance are delineated; C: Coronary angiography frame in LL projection demonstrating a single coronary artery originating from the right sinus of Valsalva (RSV) giving the left anterior descending (LAD) and continued as the circumflex artery (Lipton R- I); D: Coronary angiography frame in left anterior oblique view demonstrating a single coronary artery arising from RSV as a single unique ostium (Lipton R-III); E: Coronary angiography frame in left anterior oblique view showing a single coronary artery originating from the left sinus of Valsalva. The terminal branch of circumflex artery represented the right coronary artery (Lipton L- I). Significant stenosis of the mid circumflex artery is demonstrated (white arrow); F: Coronary angiography frame demonstrates appearance of both right and left coronary arteries on injection of left sinus of Valsalva, as a single common ostium (Lipton L- II A). Cx: Circumflex artery; RCA: Right coronary artery.

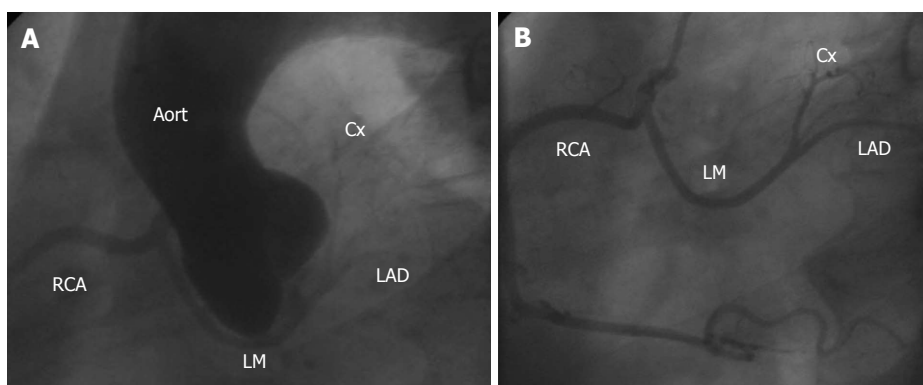


Figure 3 Angiography. A: Supravulvar aortogram in left anterior oblique projection illustrating a single origin of the coronary arteries originating from the right sinus of Valsalva (Lipton R- II A); B: Selective coronary angiography frame in left anterior oblique view showing a single coronary artery from the right sinus of Valsalva. Cx: Circumflex artery; LAD: Left anterior descending; RCA: Right coronary artery.



Figure 4 Transverse Multi-slice computed tomography scan in subtype (Lipton R- I) demonstrating the origin of the single coronary artery arising from the right sinus of Valsalva supplying the whole heart.

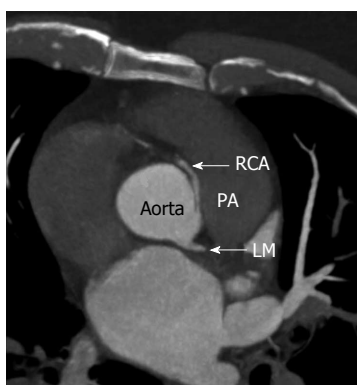


Figure 5 Volume-rendered image in subtype (Lipton L- II B) demonstrating the inter-arterial course of the right coronary artery between the aorta and pulmonary artery.

(Figures 5, 6). MSCT in patient 15 demonstrated a benign course of the SCA (Figure 7).

DISCUSSION

Historical background of classifications

The coronary arterial circulation may rarely be supplied by a SCA arising from either the right, left or posterior

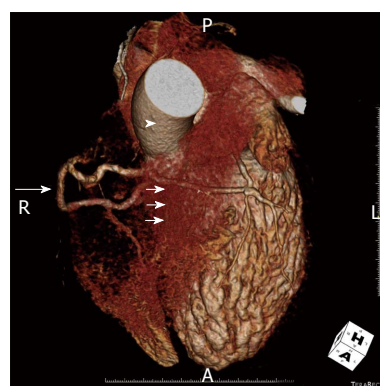


Figure 6 Three-dimensional volume-rendered image in subtype (Lipton L- II B) demonstrating the inter-arterial course of the right coronary artery (long arrow) between the aorta (arrowhead) and semitransparent pulmonary artery (short arrows).

sinus of Valsalva^[16]. The course of the SCA can be highly variable. In the last century, different classification systems for SCA based on necropsy findings and angiographic variants were suggested in the fifties by Smith^[3] (3 types), in the seventies by Lipton *et al*^[1], in the eighties by Roberts^[17], and finally through the nineties by Shirani *et al*^[2] and Roberts *et al*^[18].

Recently, a clinically useful classification scheme has been published, using either subgroups based on the site of origin and course of the anomalous coronary artery or descriptive anatomic terminology. In 2005, Rigatelli *et al*^[19,20] based his classification on clinical significance of the anomaly and launched a global practical classification of four categories (class A: benign; class B: relevant due to fixed myocardial ischemia; class C: severe, involved in sudden cardiac death (SCD); and class D: critical due to worsened clinical picture). The clinical significance and management of the various types of SCA are different as shown in Table 2.

Cheitlin *et al*^[21] expressed the pathological significance of a SCA or of both coronary arteries originating from the RSV when the anomalous artery that supplies the left coronary distribution passes leftward with an inter-arterial course between the aorta and pulmonary trunk, rendering

Table 2 Clinical-significance-based classification of coronary artery anomalies by Rigatelli *et al.*^[19,20]

Class	Subtypes	Clinical significance	Current series
A	E.g., ectopic origin of Cx from RSV ¹	Benign natural history, asymptomatic careful follow-up with conservative medical management or percutaneous intervention	Patients: none ¹
B	Ectopic origin of Cx from the RCA R- I , R- II , R- III anterior/posterior course ²	Relevant, related to myocardial ischemia Careful follow-up with conservative medical management or percutaneous intervention	Patients: 1, 2, 3, 7, 8, 9, 12, 15
C	L- I , L- II , L- III anterior/posterior course ² R- I , R- II , R- III between/interseptal course ²	Severe, potentially related to sudden cardiac death Requires surgical treatment	Patients: 6, 10, 13, 14
D	L- I , L- II , L- III between/interseptal course ² B or C subgroups with concomitant coronary atherosclerosis	Critical, class B or C with superimposed coronary artery atherosclerotic disease Requires urgent percutaneous management or surgical treatment	Patients: 4, 5, 11

¹Not included in the current paper; ²Classification according to Lipton *et al.*^[11]. Cx: Circumflex coronary artery; L: Left; R: Right; RCA: Right coronary artery; RSV: Right sinus of Valsalva.

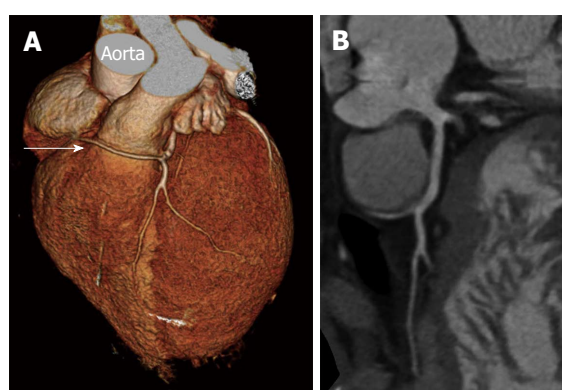


Figure 7 Single coronary artery. A: Three-dimensional volume-rendered image of benign course of right coronary artery (arrow) from left sinus of Valsalva (Lipton L-IIA); B: Transverse multi-slice computed tomography scan in subtype (Lipton L-IIA) demonstrating the origin of the single coronary artery arising from the left sinus of Valsalva supplying the whole heart.

it prone to compression and kinking on physical exercise. This variant is considered malignant since it is associated with SCD in adolescents and young adults, especially on the athletic arena. It has been found that anomalous origin of the left coronary artery from the right aortic sinus is consistently related to sudden death in more than half of the cases (53%)^[18].

On the other hand, when a SCA originating from the LSV or both coronary arteries arising from separate ostia located in the LSV with the RCA passing inter-arterially (between the aorta and pulmonary trunk, e.g., subtype Lipton L- IIB) is less deleterious even though compression can occur but SCD is a rare event. Four of our 15 patients (patients 6, 10, 13 and 14), having the above-mentioned subtype, underwent successful arterial bypass grafting to the RCA.

In a necropsy series, SCA was found in 18% of subjects. Fifty percent arose from the RSV and 50% originated from the LSV. Sudden death was twofold more frequently associated with the SCA arising from the RSV (18%) compared with those from the LSV (9%)^[12].

Coronary artery anomalies are associated with life threatening symptoms and may cause SCD during or

after strenuous exercise. The most common congenital coronary artery anomalies causing SCD involve an anomalous origin of either the right or left coronary artery arising from the left or the RSV, respectively^[22]. SCD is common (82%) when the anomalous LCA has an inter-arterial course passing between the aorta and main pulmonary artery^[12]. Moreover, SCD may rarely occur after surgical repair^[23]. The incidence is very low and estimated at 0.024% to 0.098% in the general population^[1,5,9,19,24]. The incidence of all coronary artery anomalies in the necropsy series is approximately 0.23% and varying from 0.3% to 13% in the angiographic series^[1,9,25,26]. Recently, the incidence of SCA, using dual-source computer tomography angiography, was estimated at 0.05% in the Chinese adult population^[27]. SCA may be associated with longevity and has been reported in an octogenarian^[28].

Diagnostic modalities: The correct diagnosis of a SCA and its course is not always easily made based on conventional CAG only. Precise delineation of anatomical and functional characteristics requires further complementary diagnostic modalities such as MSCT or CMR^[29-31].

Conventional CAG: Isolated SCA may be incidentally detected on routine CAG^[32], as was the case in our current series. Even with multiple projections and different angiographic views and the use of a pulmonary artery catheter, the identification of the origin and proximal course of the vessel can be difficult^[13]. Serota *et al.*^[33] proposed an angiographic technique (the dot-and-eye method) for rapid identification of the course of SCA but even with this method, identification remains difficult.

MSCT CAG: MSCT has been very useful in the diagnosis and identification of the origin and course of SCA^[28,32]. Although the radiation dose using new algorithms is decreasing, this rapidly developing non-invasive technique still has the disadvantage of radiation exposure. However, the spatial resolution (0.4-0.6 mm³) is higher than CMR and the temporal resolution of 64-slice double source MSCT is around 83 ms^[30,31,34-37]. In 5 of our patients (patients 6, 10, 13, 14 and 15) of the current series, 128-slice

MSCT confirmed the diagnosis of a SCA with clear demonstration of the inter-arterial course of the RCA originating from the LSV in four (Figure 6) and a benign course of the RCA from LSV in one (Figure 7).

Cardiovascular MR imaging: This technique has the advantage of not using ionizing radiation and has no need for the use of iodinated nephrotoxic ionic or non-ionic contrast agents. Image acquisition occurs with fairly good spatial and temporal resolution, but acquisition and imaging time is long, which makes routine use difficult and time consuming. Cardiovascular magnetic resonance proved to be useful in determining the anatomy and functional significance of SCA^[38]. Both the MSCT and the CMR imaging techniques have the additional advantage of 3-D reconstruction of the areas of the coronaries relative to the aorta and pulmonary artery. This makes a definitive diagnosis of squeezed aberrant coronary arteries between the great vessels feasible^[13].

Treatment

The detection of atherosclerotic coronary artery disease (CAD) in the presence of coronary anomalies is of practical importance, especially when a decision between PCI and CABG has to be made. For diagnostic and therapeutic reasons, the knowledge of possible variations of the coronary anatomy, their different origin, and their course is of pivotal importance. Symptomatic patients with associated significant CAD may be treated with routine interventions such as PCI or CABG^[6,39]. Angiographic recognition of coronary artery anomalies prior to surgery is of great importance. During operation, surgical complications may occur if an unrecognized anomalous vessel is excluded from perfusion during cardiopulmonary bypass or if the surgeon inadvertently damages an artery with an anomalous pathway.

Because of the reported high mortality, the occurrence of “symptomatic or asymptomatic” squeezing of SCA, regardless of the degree of atherosclerosis or site of origin, justifies arterial grafting, as was shown in 4 of our series (patients 6, 10, 13 and 14).

Significant atherosclerotic CAD^[37] in association with coronary artery anomalies has been reported in 26%-60% of cases^[1,2,40-42]. Rigatelli *et al.*^[43] suggested that benign coronary artery anomalies are not associated with or involved in the development of premature atherosclerotic CAD. Indeed the high percentage of coronary artery stenosis could be biased by the indication to perform CAG as SCA is mainly found during this diagnostic procedure. Only 4 of our 15 (27%) patients (patients 4, 5, 10 and 11) had significant CAD and 3 of them required percutaneous intervention. When the SCA does not course between the aorta and pulmonary artery, it is not vulnerable to acute angulations or kinking of the coronaries. SCA may be associated with longevity and patients in the 7th and 8th decade of life have been reported^[3,12,13,44-47], as was the case in 2 octogenarians from our current series (patients 4 and 11).

Although a SCA is often a benign congenital anomaly,

in which sudden death is a rare complication, different diagnostic modalities should be used to exclude an inter-arterial course between the aorta and pulmonary artery to detect patients at risk for serious complications.

Congenital coronary artery anomalies, detected at necropsy, associated with sudden death and without antecedent signs have been recognized in calves^[48]. SCA is not limited to the human race, it has also been reported in other mammals such as horses^[49], syrian hamsters^[50] and minipigs^[51].

As was shown in our patient's population, SCA can be associated with longevity. It has been documented up till the 8th decade of life. In the adult population, SCA-isolated or in association with acquired atherosclerotic changes-may cause severe sequelae. In some cases without CAD, the course of the SCA may be malignant.

SCA may be associated with symptomatic transient transmural myocardial ischemia, NSVT, and aborted sudden death in the absence or presence of coronary atherosclerosis. The availability of MSCT and CMR facilitates the delineation of the course of the anomalous vessel. The accurate delineation of the course of the anomalous vessel is of great importance even in patients without CAD and in cases of surgical intervention where anatomic details of the course of the vessel are of importance.

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COMMENTS

Background

Single coronary artery (SCA) is a rare congenital anomaly and occurs as an incidental finding in approximately 0.066% of the coronary angiography (CAG) population. SCA has been reported in association with and without atherosclerotic changes or in association with coronary artery fistulas, bicuspid aortic valves, and with hypertrophic cardiomyopathy.

Research frontiers

CAG is the first diagnostic tool in the detection of a SCA. Once abnormal coronary arteries are suspected, multi-slice computed tomography (MSCT) and cardiac magnetic resonance (CMR) imaging scans are excellent tools for non-invasive determination of the course of the abnormal coronaries relative to the aorta and pulmonary artery. Determination of the course of incidentally found congenital coronary anomalies during routine CAG without the direct availability of CMR or MSCT scanning is challenging.

Innovations and breakthroughs

Percutaneous coronary intervention was successfully performed in 3 patients. Eight patients were managed medically. Arterial bypass graft was performed in 4 patients with the squeezed SCA. The literature addressing SCA is reviewed.

Applications

Congenital coronary artery anomalies, detected at necropsy, associated with sudden death and without antecedent signs have been recognized in calves. SCA is not limited to the human race, it has also been reported in other mammals such as horses, syrian hamsters and minipigs.

Peer review

This paper showed that the availability and sophistications of MSCT facilitated the delineation of the course of a SCA. The authors presented a Dutch case series and review of the literature. This is an interesting report for clinical practice. Overall the report appears to be carefully examined and data adequately discussed.

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Prognostic value of increased carbohydrate antigen in patients with heart failure

Ana B Méndez, Jordi Ordoñez-Llanos, Andreu Ferrero, Mariana Noguero, Teresa Mir, Josefina Mora, Antoni Bayes-Genis, Sònia Mirabet, Juan Cinca, Eulàlia Roig

Ana B Méndez, Andreu Ferrero, Mariana Noguero, Sònia Mirabet, Juan Cinca, Eulàlia Roig, Cardiology Department, Hospital de la Santa Creu i Sant Pau, Institut de Recerca Biomèdica Sant Pau and Universitat Autònoma, 08041 Barcelona, Spain

Jordi Ordoñez-Llanos, Teresa Mir, Josefina Mora, Biochemistry Department, Hospital de la Santa Creu i Sant Pau, Institut de Recerca Biomèdica Sant Pau and Universitat Autònoma, 08041 Barcelona, Spain

Antoni Bayes-Genis, Cardiology Department, Hospital Germans Trias i Pujol, 08916 Badalona, Spain

Author contributions: Méndez AB contributed to design of the study, analysis and interpretation of data, manuscript writing and final approval of manuscript; Ordoñez-Llanos J contributed to analysis of data, review of CA125 and NT-proBNP determinations and substantial contribution to manuscript review; Ferrero A contributed to statistical assessment and substantial contribution to manuscript review; Noguero M contributed to patient selection and follow-up, Serotec and analysis of data; Mir T and Mora J contributed to CA125 and NT-proBNP determinations, analysis of data, substantial contribution to manuscript; Bayes-Genis A and Mirabet S contributed to patient selection and follow-up, analysis of data, substantial contribution to manuscript; Cinca J contributed to design of the study and substantial contribution to manuscript review; Roig E contributed to design of the study, selection of patients, analysis of data, and substantial contribution to manuscript review.

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Correspondence to: Eulàlia Roig, MD, Cardiology Department, Hospital de la Santa Creu i Sant Pau, Institut de Recerca Biomèdica Sant Pau and Universitat Autònoma, C/ Mas Casanovas 90, 08041 Barcelona, Spain. eroigm@santpau.cat
Telephone: +34-93-5565958 Fax: +34-93-5565604

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antigen 125 (CA125) and whether it adds prognostic information to N-terminal pro-brain natriuretic peptide (NT-proBNP) in stable heart failure (HF) patients.

METHODS: The predictive value of CA125 was retrospectively assessed in 156 patients with stable HF remitted to the outpatient HF unit for monitoring from 2009 to 2011. Patients were included in the study if they had a previous documented episode of HF and received HF treatment. CA125 and NT-proBNP concentrations were measured. The independent association between NT-proBNP or CA125 and mortality was assessed with Cox regression analysis, and their combined predictive ability was tested by the integrated discrimination improvement (IDI) index.

RESULTS: The mean age of the 156 patients was 72 ± 12 years. During follow-up (17 ± 8 mo), 27 patients died, 1 received an urgent heart transplantation and 106 required hospitalization for HF. Higher CA125 values were correlated with outcomes: 58 ± 85 KU/L if hospitalized vs 34 ± 61 KU/L if not ($P < 0.05$), and 94 ± 121 KU/L in those who died or needed urgent heart transplantation vs 45 ± 78 KU/L in survivors ($P < 0.01$). After adjusting for propensity scores, the highest risk was observed when both biomarkers were elevated vs not elevated (HR = 8.95, 95%CI: 3.11-25.73; $P < 0.001$) and intermediate when only NT-proBNP was elevated vs not elevated (HR = 4.15, 95%CI: 1.41-12.24; $P < 0.01$). Moreover, when CA125 was added to the clinical model with NT-proBNP, a 4% ($P < 0.05$) improvement in the IDI was found.

CONCLUSION: CA125 > 60 KU/L identified patients in stable HF with poor survival. Circulating CA125 level adds prognostic value to NT-proBNP level in predicting HF outcomes.

Abstract

AIM: To study the prognostic value of carbohydrate

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Key words: Heart failure; Prognosis; Carbohydrate antigen 125; Brain natriuretic peptides; Survival

Core tip: Increased carbohydrate antigen 125 (CA125) has prognostic implications in acute heart failure (HF). The aim of this study was to assess the prognostic value of increased CA125 and whether it adds prognostic information to N-terminal pro-brain natriuretic peptide (NT-proBNP) in stable HF patients. Higher CA125 values correlated with outcomes. The highest risk was observed when both biomarkers CA125 and NT-proBNP were elevated *vs* not elevated (HR = 8.95, 95%CI: 3.11-25.73; $P < 0.001$). CA125 > 60 KU/L identified patients in stable HF with very poor survival. Circulating CA125 level adds prognostic value to NT-proBNP level in predicting HF outcomes.

Méndez AB, Ordoñez-Llanos J, Ferrero A, Noguero M, Mir T, Mora J, Bayes-Genis A, Mirabet S, Cinca J, Roig E. Prognostic value of increased carbohydrate antigen in patients with heart failure. *World J Cardiol* 2014; 6(4): 205-212 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i4/205.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i4.205>

INTRODUCTION

Heart failure (HF) is a disease with high mortality^[1] and an estimated prevalence up to 3% in the European population^[2]. This prevalence increases exponentially with age, especially in patients older than 75 years. As the main cause of hospitalization, in patients over 65, HF is also associated with high costs. Despite recent advances in treatment, mortality is still high (12% per year) in stable patients, and there is a high rate of hospital readmissions due to worsening HF^[3,4]. Even though a high number of clinical parameters have been associated with poor outcome in patients with stable HF, the assessment of prognosis is still a challenge^[5,6]. Multiple biomarkers have been suggested to identify patients with worse prognosis, such as some interleukins, natriuretic peptides, endothelin, ST2, and several fibrosis markers^[7-11]. Although most of these effectively select patients with high risk of death, only circulating levels of natriuretic peptides have proven useful in clinical practice. Natriuretic peptides are released when ventricular wall stress and ventricular end-diastolic pressure increase. Thus, an elevated level of either brain natriuretic peptide (BNP) or the amino terminal portion of N-terminal pro-BNP (NT-proBNP) in peripheral blood is associated with decompensated HF. The negative and positive predictive values of these peptides to diagnose HF have been widely studied^[12-14]. Moreover, they are useful in the assessment of prognosis after a HF admission^[8]. However, certain limitations affect the more extensive use of natriuretic peptides^[15].

Carbohydrate antigen 125 (CA125) is a biomarker previously used in the detection and monitoring of some cancers, especially ovarian cancer^[16]. It is a high-

molecular-weight glycoprotein synthesized by epithelial cells of the serosa when there is inflammation and increased interstitial fluid; therefore, its level is elevated in the presence of pleural or pericardial effusion and ascites. Elevated CA125 has also been found in patients with HF, with or without fluid retention^[16-19]. Although its release mechanisms are not yet well understood, they correlate with increased left ventricular end-diastolic pressure, higher BNP level, and worse New York Heart Association (NYHA) functional class^[20,21]. Moreover, previous studies of BNP and CA125 in acute HF have demonstrated an additive prognostic value of CA125; therefore, the determination of both biomarkers would improve risk stratification^[22]. The advantages of CA125 determination with respect to natriuretic peptides are its higher stability in the circulation and lower cost^[23]. The usefulness of CA125 measurement to assess prognosis in patients admitted with acute decompensated HF has been previously demonstrated^[19-22]. However, information is lacking on its value in assessing the prognosis of patients with stable chronic HF. The aim of this study was to analyze the prognostic implications of increased CA125 concentration in peripheral blood and whether it adds prognostic information to NT-proBNP in stable HF patients.

MATERIALS AND METHODS

Study population

The population was a prospective cohort of 156 patients diagnosed with HF and remitted to the outpatient HF unit for monitoring from 2009 to 2011. Patients were included in the study if they had a previous documented episode of HF and received HF treatment. The diagnosis of HF was made following the Clinical Practice Guidelines of the European Society of Cardiology^[24]. HF treatment included angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs) if ACEIs were contraindicated, beta blockers, diuretics, and aldosterone antagonists, with individualized assessment of treatment indication and optimized doses per recommendations of the Clinical Practice Guidelines of the European Society of Cardiology^[24].

We excluded patients in unstable HF (NYHA functional class IV), patients with hemodynamic instability, or those diagnosed with cancer or systemic diseases that could shorten life expectancy. Patients with valve heart disease waiting for surgery repair were also excluded from the study. A 12-lead electrocardiogram (ECG) chest radiography and echocardiogram were performed in all patients to establish the HF etiology. Left ventricular systolic dysfunction was considered when ejection fraction was < 50%^[25] and ventricular hypertrophy when ventricular septum or posterior left ventricular wall thickness was > 11 mm. Ischemic heart disease was diagnosed if pathologic Q waves were observed on the ECG or significant coronary lesions on the coronary angiography. Valve heart disease was diagnosed when a biological or mechanic valve prosthesis had been implanted or if the valve dysfunction

was considered significant by echocardiography.

The hospital ethics committee approved the study, patients gave informed consent to participate, and the protocol conformed to the principles outlined in the Declaration of Helsinki.

Laboratory measurements

To obtain routine laboratory determinations and NT-proBNP concentrations, fasting serum blood samples were obtained during the first visit to the HF unit. CA125 and NT-proBNP concentrations were measured with commercial electrochemiluminescence assays in a Modular E170 analyzer (both from Roche Diagnostics, Basel, Switzerland). The CA125 assay used a pair of monoclonal anti-CA125 antibodies, one labeled with biotin, the other linked to ruthenium chelates. The analyzer had a sensitivity of 0.60 KU/L, and the total imprecision at different concentrations was $\leq 2.5\%$, expressed as a coefficient of variation.

Statistical analysis

During follow-up, mortality or need for urgent heart transplantation and new hospital admissions due to worsening HF were assessed. The primary end-point was all-cause mortality, which included need for urgent heart transplantation. The secondary end-point was worsening HF requiring hospital admission.

Continuous variables are expressed as mean \pm SD and categorical variables as percentages. Continuous variables were compared using Student's *t* test. The χ^2 or Fisher exact test was used for categorical variables. The Kruskal-Wallis test was used when necessary. Variables without homogeneous distribution were analyzed with the non-parametric Mann-Whitney test. To normalize CA125 and NT-proBNP measurements, the neperian logarithm of CA125 and NT-proBNP values was used, creating two new variables: ln-CA125 and ln-NT-proBNP. Without a clear cut-off for NT-proBNP in the literature, 3100 ng/L was selected because it was close to the mean NT-proBNP value of our population and it had the best cut-off according to area under the curve (AUC) calculation, with a sensitivity of 62% and a specificity of 82% to predict mortality. A CA125 cut-off of 60 KU/L was used, in keeping with the best predictor of mortality identified by a previous study in patients with acute HF^[22]. Based on these data, a new dichotomous variable was created for a CA125 value of 60 KU/L. Also, both variables were categorized for clinical practice and better interpretation.

To avoid overfitting multivariate models, two propensity score models^[26,27] were used to adjust for confounding factors. Propensity score analysis was performed using logistic regression for computing probability to fall in NT-proBNP 3100 ng/L or CA125 60 KU/L categories. This analysis was based on seven variables associated to end-points with the objective of eliminating differences in baseline patient characteristics that might affect event comparison. The AUC was 0.75 (0.66-0.84); $P < 0.001$ for NT-proBNP categories and 0.70 (0.61-0.80); $P < 0.001$ for CA125. Variables included in the propensity

score were left atrial diameter, age, atrial fibrillation, left ventricular ejection fraction, estimated glomerular filtrate rate (eGFR), hemoglobin and interventricular septum thickness.

The independent associations between CA125 and NT-proBNP and survival were assessed with the Cox regression analysis. The models were adjusted for the two propensity scores performed previously.

Two Cox models were compared to test improvement in risk stratification: Cox-model 1 (adjusted model with NT-proBNP 3100 ng/L) *vs* Cox-model 3 (adjusted model with 4 groups according to the cut-off concentrations of CA125 and NT-proBNP) (Table 1): The four groups in Cox model 3 were patients with both markers below the cut-off values, with only one elevated level (assessed for each biomarker), and with both markers above the cut-offs. The increase in the prognostic utility of NT-proBNP and NT-proBNP combined with CA125 when adding sequentially to the model was evaluated by the integrated discrimination improvement (IDI) index. When two nested models are compared, IDI quantifies the increment in the predicted probabilities for the subset of patients experiencing the event and the decrease for those not experiencing the event. In simpler terms, it reflects an improvement in the average of the true positive rate without sacrificing its average true negative rate^[28]. The proportionality assumption for the hazard function over time was tested by means of the Schoenfeld residuals. The discriminative ability of the final model was assessed by Harrell's *C*-statistic, and the calibration ability was assessed by the Gronnesby and Borgan test^[29]. A two-sided $P < 0.05$ was considered statistically significant for all analyses. All analyses were performed using SPSS version 19.0 (Statistical Package for the Social Sciences, Chicago, Illinois) and R (R: A Language and Environment for Statistical Computing at <http://www.R-project.org>).

RESULTS

Baseline clinical characteristics

The mean age of the studied population was 72 ± 12 years; 63% were male. Clinical characteristics of the studied population and HF treatments according to the specified 4 groups are shown in Table 1. Mean ejection fraction was $48\% \pm 17\%$, and half (77) of the patients had preserved ejection fraction. Ischemic heart disease was the most frequent etiology of HF, followed by hypertension and valve disease. The percentage of patients with HFrEF treated with ACEI/ARB was 85%, with beta-blockers 76% and with mineral corticoid receptor antagonists 56%, while in the HFpEF group the percentage of patients treated with ACEI/ARB was 69%, with beta-blockers 42% and with mineral corticoid receptor antagonists 39%. The percentage of patients treated with furosemide was similar in both groups, 84%.

Outcomes

No patient was lost during the mean follow-up of 17 ± 8 mo (range 2 to 32 mo). Monitoring of patients was blind-

Table 1 Baseline characteristics by carbohydrate antigen 125 and N-terminal pro-brain natriuretic peptide class

Variable	CA125 < 60 NT-proBNP < 3100 (n = 95, 61%)	CA125 > 60 NT-proBNP < 3100 (n = 17, 11%)	CA125 < 60 NT-proBNP > 3100 (n = 24, 15%)	CA125 > 60 NT-proBNP > 3100 (n = 20, 13%)	P
Male	55 (58%)	11 (65%)	19 (79%)	14 (70%)	0.240
Age	71 ± 12	69 ± 10	76 ± 12	73 ± 15	0.309
Hypertension	73 (77%)	10 (59%)	18 (75%)	14 (70%)	0.471
Diabetes	38 (40%)	7 (41%)	11 (46%)	8 (40%)	0.964
Dyslipidemia	46 (48%)	5 (29%)	11 (46%)	7 (35%)	0.406
Atrial fibrillation	47 (49%)	9 (53%)	17 (71%)	11 (55%)	0.316
NYHA class II	85 (89%)	7 (41%)	13 (54%)	11 (55%)	< 0.001
NYHA class III	10 (11%)	10 (59%)	11 (46%)	9 (45%)	< 0.001
HF Etiology					
Hypertension	26 (27%)	1 (6%)	5 (21%)	3 (15%)	0.212
Ischemic heart disease	30 (32%)	7 (41%)	8 (33%)	9 (45%)	0.641
Dilated cardiomyopathy	8 (8%)	2 (12%)	1 (4%)	2 (10%)	0.768
Valve heart disease	15 (16%)	5 (29%)	4 (17%)	5 (25%)	0.439
Congenital	0 (0%)	1 (6%)	1 (4%)	1 (5%)	0.058
Others	16 (17%)	1 (6%)	5 (21%)	0 (0%)	0.110
Systolic BP (mmHg)	129 ± 18	118 ± 20	119 ± 21	114 ± 12	0.001
Diastolic BP (mmHg)	74 ± 12	67 ± 8	73 ± 14	71 ± 9	0.156
LVEF (%)	51 ± 16	46 ± 16	46 ± 22	38 ± 18	0.015
LVDD (mm)	54 ± 8	52 ± 9	56 ± 13	57 ± 11	0.290
LAD (mm)	49 ± 9	49 ± 9	51 ± 9	54 ± 12	0.184
IVS (mm)	12 ± 3	12 ± 2	13 ± 3	11 ± 3	0.158
LVPW (mm)	11 ± 2	10 ± 2	11 ± 2	10 ± 2	0.461
Na ⁺ (mEq/dL)	140 ± 3	138 ± 4	140 ± 4	139 ± 5	0.227
K ⁺ (mEq/dL)	4.2 ± 0.5	4.3 ± 0.6	4.4 ± 0.7	4.2 ± 0.5	0.453
GF (mL/min per 1.73 m ²)	61 ± 20	62 ± 16	49 ± 22	53 ± 20	0.029
Hemoglobin (g/dL)	131 ± 20	118 ± 25	126 ± 21	129 ± 24	0.111

LVEF: Left ventricular ejection fraction; NYHA: New York Heart Association; HF: Heart failure; LVDD: Left ventricular diastolic diameter; LAD: Left auricular diameter; IVS: Interventricular septum; LVPW: Left ventricular posterior wall; BP: Blood pressure; Na: Sodium; K: Potassium; GF: Glomerular filtration; CA125: Carbohydrate antigen 125; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

Table 2 Univariate analysis, variables significantly associated with mortality or need for urgent cardiac transplantation

Variable	Deaths	Alive	P
Age	78 ± 12	70 ± 12	0.004
LVEF (%)	53 ± 16	47 ± 17	0.08
IVS (mm)	13.7 ± 3.4	11.9 ± 2.5	0.005
LA (mm)	54 ± 12	49 ± 9	0.01
GF (MDRD)	46 ± 18	61 ± 19	0.0001
Hemoglobin (g/L)	121 ± 21	130 ± 21	0.02
Na ⁺ (mEq/mL)	140 ± 4	139 ± 3	0.54
K ⁺ (mEq/mL)	4.3 ± 0.6	4.2 ± 0.5	0.54
Systolic BP (mmHg)	120 ± 19	125 ± 19	0.24
Diastolic BP (mmHg)	70 ± 12	73 ± 12	0.25
CA125 (KU/L)	94 ± 121	45 ± 78	0.01
NT-proBNP (pg/dL)	6613 ± 8437	2326 ± 2823	0.02
AF (%)	71	50	0.05
NYHA class III (%)	70	30	0.03

LVEF: Left ventricular ejection fraction; IVS: Interventricular septum; LA: Left atrial; GF: Glomerular filtration; Na: Sodium; K: Potassium; BP: Blood pressure; CA125: Carbohydrate antigen 125; NT-proBNP: N-terminal pro-B-type natriuretic peptide; AF: Atrial fibrillation; NYHA: New York Heart Association; MDRD: Modification of diet in renal disease.

ed to CA125 values. During follow-up, 27 patients died (17%), and 1 had progressive HF deterioration requiring urgent heart transplantation at one year. All 27 deaths were due to cardiovascular causes, 23 (85%) of them to progressive HF (3 of which required hospital admission

for decompensated HF) and 3 to cerebrovascular accident. The remaining patient had a diagnosis of ischemic heart disease and was lost to sudden death while being treated with chemotherapy for rectal cancer that appeared during follow-up. Mortality was similar for women (19%) and men (18%). There was a high incidence of worsening, new HF episodes: 106 patients required an admission for decompensated HF (68%), and nearly half (50 patients) were admitted more than once for HF during follow-up.

Prognostic implications of CA125

Older age, increased ventricular septum thickness and left atrial diameter, low eGFR and hemoglobin concentration, higher CA125 and NT-proBNP levels, the presence of atrial fibrillation, and worse NYHA functional class were all associated with mortality or need for urgent heart transplantation by univariate analyses (Table 2). Hospitalization for worsening HF was associated with low eGFR, higher CA125 and NT-proBNP values, presence of atrial fibrillation, and worse NYHA functional class by univariate analyses (Table 3). Patients who died and those who required admission for decompensated HF had significantly lower eGFR, had higher CA125 and NT-proBNP, and more frequently had atrial fibrillation and NYHA functional class III. In univariate analyses, ln-CA125 was positively associated with ln-NT-proBNP ($\beta = 0.39$, $P =$

Table 3 Univariate analysis, variables significantly associated with need for hospitalization for decompensated heart failure

	Hospitalization	No hospitalization	P
LVEF (%)	48 ± 18	49 ± 17	0.7
IVS (mm)	12 ± 3	11 ± 3	0.14
LA (mm)	50 ± 9	48 ± 10	0.15
GF (MDRD)	56 ± 19	64 ± 21	0.01
Hemoglobin (g/L)	127 ± 22	132 ± 20	0.16
Na ⁺ (mEq/mL)	139 ± 3.7	140 ± 3.6	0.4
K ⁺ (mEq/mL)	4.2 ± 0.5	4.3 ± 0.6	0.3
Systolic BP (mmHg)	124 ± 18	125 ± 20	0.9
Diastolic BP (mmHg)	73 ± 11	72 ± 13	0.7
CA125 (KU/L)	58 ± 85	34 ± 61	0.01
NT-proBNP (pg/dL)	3431 ± 4792	2031 ± 3234	0.03
AF (%)	61	39	0.01
NYHA class III (%)	97	3	0.0001

LVEF: Left ventricular ejection fraction; IVS: Interventricular septum; LA: Left atrial; GF: Glomerular filtration; Na: Sodium; K: Potassium; BP: Blood pressure; CA125: Carbohydrate antigen 125; NT-proBNP: N-terminal pro-B-type natriuretic peptide; AF: Atrial fibrillation; NYHA: New York Heart Association.

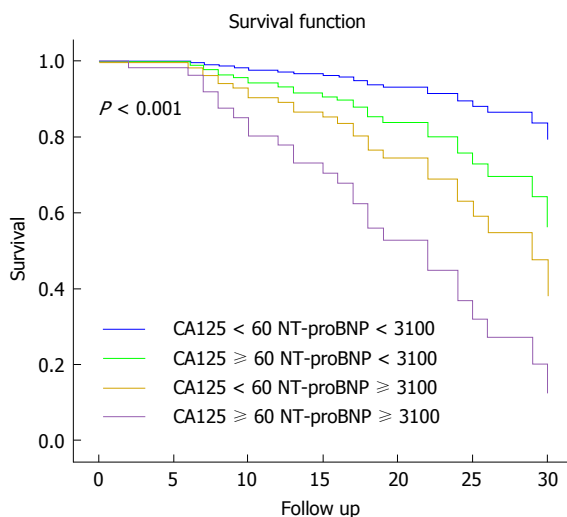


Figure 1 Kaplan-Meier survival curves in the 4 groups defined by N-terminal pro-brain natriuretic peptide < or ≥ 3100 ng/L and carbohydrate antigen 125 < or ≥ 60 KU/L. Patients with both biomarkers elevated presented the worst survival. CA125: Carbohydrate antigen 125; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

0.0001) and negatively with left ventricular ejection fraction ($\beta = -0.23$, $P = 0.003$) and sodium concentration ($\beta = -0.24$, $P = 0.003$). There was no relationship between ln-CA125 and eGFR or hemoglobin. The receiver operating characteristic curve analysis showed similar values for CA125 and NT-proBNP; the AUC for mortality prediction was 0.699 (95%CI: 0.59-0.80) for CA125 and 0.710 (95%CI: 0.59-0.82) for NT-proBNP.

The relationship between CA125 and outcomes was evaluated in multivariate Cox proportional analyses. Cox-model 1 identified NT-proBNP ≥ 3100 ng/L and Cox-model 2 identified CA125 ≥ 60 KU/L as independent predictors of mortality (Table 4). Two Cox models were compared to test improvement in risk stratification: Cox-model 1 (adjusted model with NT-proBNP ≥ 3100 ng/L)

Table 4 Multivariate analysis for global mortality

	HR	95%CI	Sig
Cox-model 1 variable			
NT-proBNP (3100 ng/L)	4.95	2.11-11.62	< 0.001
Cox-model 2 variable			
CA125 (60 KU/L)	3.32	1.50-7.37	0.003
Cox-model 2 variable			
CA125 < 60 NT-proBNP < 3100	1		
CA125 > 60 NT-proBNP < 3100	2.49	0.65-9.53	0.18
CA125 < 60 NT-proBNP > 3100	4.15	1.41-12.24	< 0.01
CA125 > 60 NT-proBNP > 3100	8.95	3.11-25.73	< 0.001

Adjusted by propensity scores of NT-proBNP > 3100 ng/L and CA125 > 60 KU/L categories. CA125: Carbohydrate antigen 125; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

vs Cox-model 3 (adjusted model with 4 groups according to the cut-off concentrations of CA125 and NT-proBNP). Cox-model 1 had good discriminative ability (Harrell's C -statistic = 0.774), and Cox-model 3 had slightly better discriminative ability (Harrell's C -statistic = 0.777) and good calibration ability ($P = 0.39$, Gronnesby and Borgan test). However, the C -statistic is not sensitive to changes when a new factor is included in a model. Therefore, when we compared Cox-model 1 vs Cox-model 3 by the IDI index, the discrimination slope of the Cox-model 3 was 4 percentage points higher than model 1 ($P < 0.05$). Survival curves adjusted by propensity scores from Cox-model 3 (Figure 1) showed risk stratification when HF patients were classified into the 4 groups defined by NT-ProBNP and CA125 cut-offs. Patients with both biomarkers elevated presented the worst survival.

DISCUSSION

In our study, a concentration of CA125 > 60 KU/L was identified as an independent predictor of mortality or of the need for urgent heart transplantation at mid-term follow-up. This biomarker also added prognostic information beyond that provided by NT-proBNP concentration. Patients with NT-proBNP ≥ 3100 ng/L and CA125 ≥ 60 KU/L had extremely low survival during follow-up.

CA125 and HF

CA125 is a cancer marker that can be synthesized in mesothelial cells from the peritoneum and pleura^[30]. CA125 concentration is increased in patients with pericardial, pleural, and peritoneal effusions^[30]. Increased CA125 concentration in patients with HF is believed to be multifactorial and secondary to the increased end-diastolic and pulmonary venocapillary pressure that causes interstitial pulmonary edema^[31], an inflammatory substrate present in HF. In addition, the appearance of pleural effusion and signs of congestion have been associated with increased CA125^[32].

The good correlation of CA125 with natriuretic peptides suggests a similar release mechanism related to an increase in intracavitary pressures and stress of both atrial and ventricular walls. It has also been suggested that

mesothelial cells can secrete CA125 in response to activation of cytokines. Thus, the activation of either TNF- α or interleukin-6 has been associated with elevated CA125, and the increase of these cytokines correlates with the severity of HF^[7,18]. It has been suggested that increased CA125 can be a sign of both congestion and inflammation; both parameters have been associated with poor prognosis in HF patients. Furthermore, due to the long half-life of CA125 (about 12 d) it can be used as an indirect marker of fluid retention in the weeks preceding blood extraction, in both acute and chronic HF. Especially in patients with stable HF, CA125 ≥ 60 KU/L can be an early sign of congestion that would identify patients with worse prognosis.

Previous studies

Numerous studies have examined the value of natriuretic peptides to assess the prognosis of HF^[33-36], most of them performed in the emergency room or in patients with acute decompensated HF requiring hospitalization. Few of the studies focus on stable chronic HF^[37,38]. Earlier studies in acute HF patients associated increased CA125 with worse NYHA functional class, greater left atrial size, higher BNP, and poor prognosis at 6 mo follow-up^[19,20,22]. A recent study by Ordu *et al.*^[37] reports that increased CA125 and NT-proBNP were similar in their capacity to predict prognosis in patients with stable HF^[38]. Although our population differed from theirs, which included only patients with systolic dysfunction and elevated NT-proBNP, the CA125 concentrations were similar in both studies. Our patients were better controlled, with higher proportions receiving ACEI/ARB, beta-blockers and spironolactone therapy. Both studies report a significant correlation between CA125 and NT-proBNP. However, Ordu *et al.*^[37] used a higher NT-proBNP cut-off value for patient stratification than previous studies^[11,15]. It is possible that with this high cut-off value the addition of CA125 did not improve upon the predictive value obtained with only NT-proBNP. However, lower NT-proBNP is frequently found in stable HF patients. In our study, CA125 ≥ 60 KU/L added prognostic information to that obtained from the ≥ 3100 ng/L NT-proBNP cut-off. In fact, when both biomarkers were elevated, the prognosis was very poor. In another recent study performed in stable patients with left ventricular dysfunction, a value of CA125 ≥ 60 KU/L was associated with an increased risk of cardiovascular death and hospitalization for HF^[22]. Our study did not select patients according to the degree of left ventricular dysfunction; all patients diagnosed with HF were included. A high level of CA125 allowed the identification of patients with worse prognosis independently of their ejection fraction. This is important because almost half of all patients diagnosed with HF in the majority of epidemiological studies have preserved ejection fraction^[39]. Furthermore, new biomarkers are under development to improve diagnosis and prognosis assessment in HF. Recently, experimental studies have suggested that changes in circulating microRNAs can be used as a biomarker of

disease^[40,41]. However, these new molecular markers are still under investigation. On the contrary, more than 10 years of experience with natriuretic peptides and CA125 have already been reported^[12,20].

Although the value of natriuretic peptides for the diagnosis of HF has been widely demonstrated, its usefulness to assess prognosis in some cases remains controversial. Thus, in advanced HF, there is controversy whether BNP concentration helps identify patients requiring heart transplantation^[42,43]. Similarly, in asymptomatic individuals with HF and those in pre-HF stages, NT-proBNP failed to predict the patients who presented with clinical HF during follow-up^[44]. Other limitations to the use of natriuretic peptides exist, particularly with NT-proBNP since cut-off points for assessing prognosis have not been clearly established. Therefore, the addition of CA125 may help to improve prognosis assessment in patients with stable HF.

Limitations

In this study the inclusion criterion was the diagnosis of HF. The predictive value of CA125 possibly could have been even greater if left ventricular dysfunction had been a selection criterion. Although the cohort was relatively small, mortality and hospitalization rates were similar to previous studies analyzing stable HF patients. When the study population was divided according to CA125 and NT-proBNP values, some subgroups were quite small. Nonetheless, increased CA125 concentration was effective in identifying stable HF patients at high risk of death and new admissions for worsening HF, and despite having a relatively small sample size the results are consistent with those obtained in previous studies in acute HF.

In conclusion, CA125 is an excellent marker of prognosis in patients with stable HF. CA125 ≥ 60 KU/L identified patients at high risk of death or need for urgent heart transplantation. CA125 added prognostic information to the predictive value of NT-proBNP. Its easy determination and low cost may encourage its expanded use.

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COMMENTS

Background

Heart failure (HF) is the final phase of many heart diseases. The estimated prevalence is up to 3% in the European population. This prevalence increases exponentially with age. As the main cause of hospitalization, in patients over 65, HF is also associated with high costs. Despite recent advances in treatment, mortality is still high (12% per year) in stable patients, and there is a high rate of hospital readmissions due to worsening HF. Even though a high number of clinical parameters have been associated with poor outcome in patients with stable HF, the assessment of prognosis is still a challenge.

Research frontiers

Several biomarkers have been used to assess the prognosis of patients with HF. Although the natriuretic peptides are considered the most widely used bio-

markers, they still have some limitations. Increased concentrations of natriuretic peptides have been associated with worse prognosis in patients hospitalized for worsening HF. However, its utility to identify patients at high risk of death in stable HF has been less studied. Furthermore, although new molecular biomarkers are under development to improve prognosis assessment in HF, they are not ready for clinical use. On the other hand, more than 10 years of experience with natriuretic peptides and carbohydrate antigen 125 (CA125) have already been reported.

Innovations and breakthroughs

This study provides the results of a clinical investigation demonstrating that the combination of two well-known biomarkers, CA125 and N-terminal pro-brain natriuretic peptide (NT-proBNP), is useful to select patients with stable HF and poor outcome. CA125 concentration, when added to NT-proBNP, provides relevant prognostic information.

Applications

CA125 concentration can be routinely added to NT-proBNP assessment in stable HF patients, at a low cost, to identify patients at high risk of death or a worsening HF episode.

Terminology

CA125 is a biomarker previously used in the detection and monitoring of some cancers. It is a high-molecular-weight glycoprotein synthesized by epithelial cells of the serosa when there is inflammation and increased interstitial fluid. Elevated CA125 has also been found in patients with HF, with or without fluid retention.

Peer review

The authors report on the prognostic value of CA125 in patients with stable chronic HF. This biomarker has proven its validity in previous studies, in this manuscript has additive value, due to the combination of the new biomarker with the established brain natriuretic peptide-value.

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Cardiac embolism after implantable cardiac defibrillator shock in non-anticoagulated atrial fibrillation: The role of left atrial appendage occlusion

Xavier Freixa, Rut Andrea, Victoria Martín-Yuste, Diego Fernández-Rodríguez, Salvatore Brugaletta, Mónica Masotti, Manel Sabaté

Xavier Freixa, Rut Andrea, Victoria Martín-Yuste, Diego Fernández-Rodríguez, Salvatore Brugaletta, Mónica Masotti, Manel Sabaté, Department of Interventional Cardiology, Thorax Institute, Hospital Clinic of Barcelona, University of Barcelona, 08036 Barcelona, Spain

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Correspondence to: Xavier Freixa, MD, Department of Interventional Cardiology, Thorax Institute, Hospital Clinic of Barcelona, University of Barcelona, c/Villarroel 170, 08036 Barcelona, Spain. xavierfreixa@hotmail.com

Telephone: +34-93-2275519 Fax: +34-93-2275505

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risk. Although LAA occlusion is a relatively new technique, its usage is rapidly expanding worldwide and constitutes a very valid alternative for patients with NVAf and a formal contraindication to OAC.

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Key words: Left atrial appendage; Implantable cardiac defibrillator; Defibrillator; Atrial fibrillation

Core tip: The present case report discusses the treatment of a patient with atrial fibrillation and contraindication to anticoagulation who presented with a massive peripheral embolism after an implantable cardiac defibrillator shock. The manuscript describes the successful management of the patient and discusses a clinical setting that might be associated with an increased cardioembolic risk.

Abstract

Cardioembolic events are one of the most feared complications in patients with non-valvular atrial fibrillation (NVAf) and a formal contraindication to oral anticoagulation (OAC). The present case report describes a case of massive peripheral embolism after an implantable cardiac defibrillator (ICD) shock in a patient with NVAf and a formal contraindication to OAC due to previous intracranial hemorrhage. In order to reduce the risk of future cardioembolic events, the patient underwent percutaneous left atrial appendage (LAA) occlusion. A 25 mm Amplatzer™ Amulet was implanted and the patient was discharged the following day without complications. The potential risk of thrombus dislodgement after an electrical shock in patients with NVAf and no anticoagulation constitutes a particular scenario that might be associated with an additional cardioembolic

Freixa X, Andrea R, Martín-Yuste V, Fernández-Rodríguez D, Brugaletta S, Masotti M, Sabaté M. Cardiac embolism after implantable cardiac defibrillator shock in non-anticoagulated atrial fibrillation: The role of left atrial appendage occlusion. *World J Cardiol* 2014; 6(4): 213-215 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i4/213.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i4.213>

INTRODUCTION

Cardioembolic events are one of the most feared complications in patients with non-valvular atrial fibrillation (NVAf) and a formal contraindication to oral anticoagulation (OAC). In these patients, the risk of stroke can generally be predicted using the CHA₂DS₂-VASc score^[1]. However, factors not contemplated in the CHA₂DS₂-

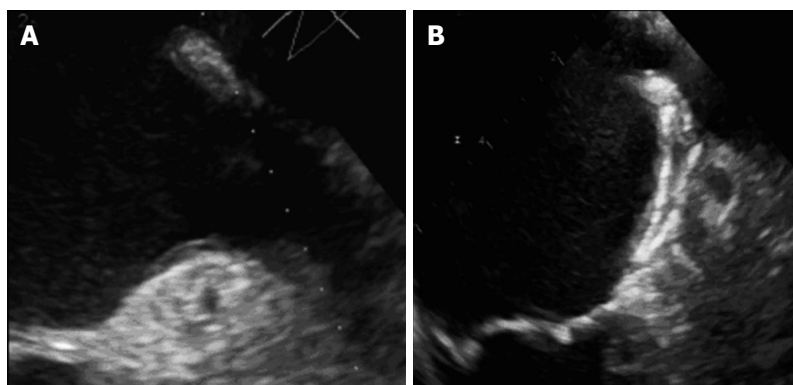


Figure 1 Left atrial appendage before and after occlusion with an Amplatzer Amulet. Left atrial appendage before (A) and after (B) percutaneous occlusion.

VASc score may also play a relevant role. One of these factors might be the presence of implantable cardiac defibrillators (ICD) and the potential risk of thrombus dislodgement after electrical shocks. In the following report, we describe a case of massive peripheral embolism after an ICD shock in a patient with NVAF and a formal contraindication to OAC. In order to reduce the risk of new cardioembolic events, the patient underwent percutaneous left atrial appendage (LAA) occlusion. Although LAA occlusion is a relatively new technique, its usage is rapidly expanding worldwide and constitutes a valid alternative for patients with NVAF and a formal contraindication to OAC.

CASE REPORT

This was a 61-year-old male with a previous history of hypertension, diabetes, stroke, dilated cardiomyopathy and ICD for secondary prevention. The patient also presented chronic NVAF with a CHA₂DS₂VASc of 5 treated initially with OAC. Anticoagulation was, however, discontinued after an episode of intracranial hemorrhage and single aspirin treatment was started. Six months after OAC discontinuation, the patient was admitted with a massive abdominal embolism after an appropriate ICD shock requiring mechanical aspiration of emboli in the right hepatic and superior mesenteric arteries. After consultation with the neurology department, reintroduction of OAC was not recommended as a result of the risk of recurrent intracranial bleeding. Transesophageal echocardiography (TEE) showed no thrombus in the LAA and a mean diameter of 22 mm at the landing area. Considering the high risk of thrombus formation as a result of the slow LAA blood flow velocity (0.3 m/s) and the risk of cardioembolic recurrence after another potential ICD shock, percutaneous LAA occlusion with a 25 mm Amplatzer™ Amulet™ was conducted without complications (Figure 1). The patient was discharged the following day under dual antiplatelet therapy. At 3 mo, TEE showed complete LAA sealing and the patient was left on single antiplatelet therapy again.

DISCUSSION

In patients with NVAF, cardioembolic strokes are gener-

ally more disabling and more lethal than strokes from other sources^[2]. Although OAC has been shown to be highly effective in reducing the rate of cardioembolic events and deaths^[3], between 30% and 50%^[4] of patients present a formal contraindication for OAC, have unstable international normalized ratios or are not fully compliant. Currently, percutaneous LAA occlusion represents a valid alternative in patients with NVAF and a formal contraindication for OAC, but it might also be considered for those at high risk of bleeding or drug cessation (II-b indication)^[5]. Although the presence of an ICD is not contemplated in the CHA₂DS₂VASc score, the authors believe that it should be taken into consideration when assessing the cardioembolic risk in patients with NVAF and no anticoagulation. In fact, the incidence of cardioembolic events after electrical shocks remains high in these patients, ranging between 5% and 7% with every shock^[6]. In addition, the CHA₂DS₂VASc score in patients with ICDs is generally high as a result of the increased cardiovascular comorbidity. The usual high CHA₂DS₂VASc score of this population, the unpredictable formation of thrombus in the LAA without anticoagulation, and the increased risk of thrombus dislodgement after ICD shocks constitute a particular scenario that might be associated with a high risk of cardioembolic events. In this sense, the occlusion of the LAA, an anatomical structure related with most cardioembolic events, might be a valid alternative. Although further evidence will be necessary to determine if the presence of ICD constitutes an independent predictor of cardioembolic events in patients with NVAF and the absence of OAC, the present case report is hypothesis generating as it highlights the specific risk of these patients and describes a potential alternative for their management.

COMMENTS

Case characteristics

Secondary prevention for cardiac embolism in a patient with previous peripheral embolism and non-anticoagulated atrial fibrillation.

Clinical diagnosis

Non-anticoagulated atrial fibrillation in a patient with previous implantable cardiac defibrillator and cardiac embolism after electrical shock.

Differential diagnosis

The potential risk of thrombus dislodgement after an electrical shock in patients with atrial fibrillation and no anticoagulation constitutes a particular scenario

that might be associated with an additional cardioembolic risk.

Imaging diagnosis

Previous intracranial hemorrhage.

Pathological diagnosis

Non-valvular atrial fibrillation (NVAf) with formal contraindication to anticoagulation due to previous intracranial bleeding.

Treatment

Percutaneous left atrial appendage (LAA) occlusion.

Experiences and lessons

Although LAA occlusion is a relatively new technique, its usage is rapidly expanding worldwide and constitutes a valid alternative for patients with atrial fibrillation and a formal contraindication to oral anticoagulation (OAC).

Peer review

The authors reported a case with non-valvular atrial fibrillation and a formal contraindication to OAC, who had undergone percutaneous LAA occlusion after the occurrence of massive abdominal embolism. This case report is interesting and suggestive but there are several questions to be solved.

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World Journal of *Cardiology*

World J Cardiol 2014 May 26; 6(5): 216-352



**TOPIC HIGHLIGHT**

- 216 Are there ways to attenuate arterial calcification and improve cardiovascular outcomes in chronic kidney disease?
Vo TM, Disthabanchong S
- 227 Management of hypertension in primary aldosteronism
Aronova A, Fahey III TJ, Zarnegar R
- 234 Anti-hypertensive drugs in children and adolescents
Chu PY, Campbell MJ, Miller SG, Hill KD
- 245 Alcohol-induced hypertension: Mechanism and prevention
Husain K, Ansari RA, Ferder L
- 253 Pediatric hypertension: An update on a burning problem
Bassareo PP, Mercurio G
- 260 Potential pathophysiological role for the vitamin D deficiency in essential hypertension
Carbone F, Mach F, Vuilleumier N, Montecucco F
- 277 Device-guided breathing exercises for the treatment of hypertension: An overview
van Hateren KJ, Landman GW, Logtenberg SJ, Bilo HJ, Kleefstra N
- 283 Hypertension and chronic ethanol consumption: What do we know after a century of study?
Marchi KC, Muniz JJ, Tirapelli CR
- 295 Asserted and neglected issues linking evidence-based and Chinese medicines for cardiac rehabilitation
Ferreira AS, Moura NGR

REVIEW

- 304 Alterations in cell adhesion proteins and cardiomyopathy
Li J
- 314 Autoantibodies to apolipoprotein A-1 as a biomarker of cardiovascular autoimmunity
Vuilleumier N, Montecucco F, Hartley O

- | | | |
|--------------------------|-----|--|
| ORIGINAL ARTICLE | 327 | Elevated blood pressure: Our family's fault? The genetics of essential hypertension
<i>Natekar A, Olds RL, Lau MW, Min K, Imoto K, Slavin TP</i> |
| SYSTEMATIC REVIEW | 338 | Heart and lung, a dangerous liaison-Tako-tsubo cardiomyopathy and respiratory diseases: A systematic review
<i>Manfredini R, Fabbian F, De Giorgi A, Pala M, Mallozzi Menegatti A, Parisi C, Misurati E, Tiseo R, Gallerani M, Salmi R, Bossone E</i> |
| CASE REPORT | 345 | Long-lasting symptoms and diagnostics in a patient with unrecognized right sided heart failure: Why listening to the heart is so important
<i>de Vette LC, Brugts JJ, McGhie JS, Roos-Hesselink JW</i> |
| | 349 | Unreliability of aortic size index to predict risk of aortic dissection in a patient with Turner syndrome
<i>Nijs J, Gelsomino S, Lucà F, Parise O, Maessen JG, La Meir M</i> |

Contents

World Journal of Cardiology
Volume 6 Number 5 May 26, 2014

APPENDIX I-V Instructions to authors

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WJC 6th Anniversary Special Issues (1): Hypertension

Are there ways to attenuate arterial calcification and improve cardiovascular outcomes in chronic kidney disease?

Thanh-Mai Vo, Sinee Disthabanchong

Thanh-Mai Vo, Division of Nephrology, Saint Louis University, Saint Louis, MO 63110, United States

Sinee Disthabanchong, Division of Nephrology, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

Author contributions: Vo TM and Disthabanchong S equally contributed to this paper.

Correspondence to: Sinee Disthabanchong, MD, Associate Professor of Medicine, Division of Nephrology, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, 270 Rama VI Rd, Phayathai, Bangkok 10400, Thailand. sineemd@hotmail.com

Telephone: +66-2-2011116 Fax: +66-2-2011400

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Abstract

The risk of cardiovascular mortality among patients with end-stage renal disease is several times higher than general population. Arterial calcification, a marker of atherosclerosis and a predictor of cardiovascular mortality, is common in chronic kidney disease (CKD). The presence of traditional cardiovascular risk factors such as diabetes, hypertension, hyperlipidemia, and advanced age cannot fully explain the high prevalence of atherosclerosis and arterial calcification. Other factors specific to CKD such as hyperphosphatemia, excess of calcium, high dose active vitamin D and prolonged dialysis vintage play important roles in the development of arterial calcification. Due to the significant health risk, it is prudent to attempt to lower arterial calcification burden in CKD. Treatment of hyperlipidemia with statin has failed to lower atherosclerotic and arterial calcification burden. Data on diabetes and blood pressure controls as well as smoking cessation on cardiovascular outcomes in CKD population are limited. Currently available treatment options include non-calcium

containing phosphate binders, low dose active vitamin D, calcimimetic agent and perhaps bisphosphonates, vitamin K and sodium thiosulfate. Preliminary data on bisphosphonates, vitamin K and sodium thiosulfate are encouraging but larger studies on efficacy and outcomes are needed.

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Key words: Vascular calcification; Coronary calcification; Hemodialysis; Dialysis; Chronic kidney disease

Core tip: Arterial calcification is common in chronic kidney disease (CKD). Factors specific to CKD such as hyperphosphatemia, excess of calcium and high dose vitamin D therapy play important roles in the development of arterial calcification. Statin is ineffective in lowering the calcification burden. Data on diabetes and blood pressure controls and smoking cessation on cardiovascular outcomes in CKD population are limited. Available treatment strategies include non-calcium containing phosphate binders, low dose active vitamin D and calcimimetic agent. Preliminary data on bisphosphonates, vitamin K and sodium thiosulfate are encouraging but larger studies on efficacy and outcomes are needed.

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INTRODUCTION

Cardiovascular disease is the leading cause of death in

chronic kidney disease (CKD) population. The risk of cardiovascular mortality among those with end-stage renal disease is several times higher than general population^[1]. Arterial calcification, a marker of atherosclerosis and a predictor of cardiovascular mortality, is common in CKD^[2]. The presence of arterial calcification leads to an increase in arterial stiffness and a decrease in coronary perfusion resulting in cardiac hypertrophy and myocardial ischemia. Young adults who have been on hemodialysis for a long period of time have the prevalence of coronary artery calcification (CAC) that is at least ten times higher than those of the same age whose kidney function are normal^[3]. Moreover, an inverse relationship between the estimated glomerular filtration rate and the degree of CAC was observed^[4]. The presence of traditional cardiovascular risk factors such as diabetes, hypertension, hyperlipidemia, and advanced age cannot fully explain the high prevalence of atherosclerosis and arterial calcification in CKD^[5]. Along with common cardiovascular risk factors, other factors specific to CKD population such as hyperphosphatemia, excess of calcium from calcium-containing phosphate binders and high calcium concentration in dialysis solution, high dose active vitamin D used in the treatment of hyperparathyroidism and prolonged dialysis vintage have been shown to positively influence the development of arterial calcification^[3,6].

MINERAL METABOLISM IN CKD

In early CKD, the kidney's ability to excrete phosphate load is impaired resulting in a release of fibroblast growth factor 23 (FGF-23) whose action is to stimulate renal phosphate excretion in order to maintain neutral phosphate balance^[7]. FGF-23, in the presence of its obligatory co-receptor klotho, binds to FGF receptors causing a decrease in phosphate reabsorption in the proximal tubules and a suppression of 1,25 dihydroxyvitamin D (1,25-OH₂-D) synthesis^[8]. Continued phosphate retention and decreased 1,25-OH-D levels later on lead to an increase in parathyroid hormone (PTH) secretion. The accumulation of FGF-23 together with PTH work in concert to enhance renal phosphate excretion. As CKD advances, these compensatory mechanisms fail and phosphate retention ensues evidenced by the development of hyperphosphatemia^[9]. Accumulation of PTH also enhances bone resorption giving rise to an increase in circulating calcium, bone loss and fracture. On the other hand, elevated FGF-23 has been linked to cardiac hypertrophy, vascular calcification, congestive heart failure and increased mortality^[10-13].

PATHOGENESIS OF ARTERIAL CALCIFICATION

Pathogenesis of arterial calcification is no longer believed to be the passive precipitation of calcium and phosphate crystals but involves a tightly regulated process of cellular transformation of vascular smooth muscle cells (VSMCs) into osteoblast-like cells. These calcified VSMCs, instead

of retaining smooth muscle cell markers, express specific osteoblast markers as well as several bone matrix proteins^[14,15]. The process of calcification also has features that resemble bone matrix mineralization. For example, the formation and nucleation of mineral crystals require the presence of matrix vesicles. Dying VSMCs form apoptotic bodies which have the ability to concentrate calcium and phosphate in the same fashion as matrix vesicles^[16]. Several factors related to CKD including high calcium and phosphate environment and high dose active vitamin D have been shown to promote VSMC transformation followed by matrix vesicle-mediated mineralization^[17,18]. Moreover, the reduction and the alteration of function of naturally occurring calcification inhibitors such as fetuin A, matrix gla-protein, osteopontin and osteoprotegerin are also important in the development of arterial calcification in CKD^[19,20]. Klotho deficiency has been observed in kidneys, parathyroid glands and other organs during the course of CKD^[21,22]. In arterial wall, decreased klotho expression potentiates the development of arterial calcification^[23,24]. The role of FGF-23 in arterial calcification is complex. Few studies have identified FGF receptor and its signaling pathway in the arterial wall whereas others have not^[12,23,25]. Kidney transplantation can markedly improve both renal function and mineral metabolism in the long term. Several studies have demonstrated stabilization or decline in the rate of progression of arterial calcification in patients who received a kidney transplant as compared to those who remained on dialysis especially during the first 1-2 years^[26-28]. However, with longer follow-up period up to 3-4 years post-transplantation, the progression becomes more evident. Overall the rate of CAC progression was estimated to be around 10% per year^[29,30]. The severity of baseline calcification and the presence of hyperlipidemia were identified as independent predictors of progression in these studies. It appears that once calcification develops it probably cannot be reversed. Despite the significant improvement in kidney function and mineral metabolism, arterial calcification tends to become more severe as time passes probably triggering by the presence of common cardiovascular risk factors in kidney transplant recipients including aging, diabetes, hypertension and hyperlipidemia.

Due to the significant health risk of atherosclerosis and arterial calcification, it is prudent to attempt to lower calcification burden in CKD patients. Cardiovascular risk modification through the use of statin for hyperlipidemia has not been proved fruitful in attenuating CAC progression^[31,32]. Studies on diabetes and blood pressure controls as well as smoking cessation on cardiovascular outcomes in CKD population are limited. The following review focuses on therapies that can modify CKD-related risk factors for arterial calcification which may have favorable impact on cardiovascular outcomes.

PHOSPHATE BINDERS

The purpose of phosphate binder is to bind phosphate in the ingested food and increase its elimination in the

stool. Calcium-containing phosphate binders such as calcium carbonate and calcium acetate are commonly used as phosphate binding agents since early 1980s as alternatives to aluminum hydroxide due to the high prevalence of aluminum toxicity. The use of calcium-containing phosphate binders is often limited by the development of hypercalcemia. Furthermore, over the past decade, increasing evidence have linked the amount of calcium intake derived from calcium-containing phosphate binders to the severity of vascular calcification^[3,6,33]. Newer phosphate binding agents including sevelamer, lanthanum, calcium-magnesium combination and iron-based phosphate binders have been developed to overcome these limitations.

Sevelamer carbonate

Sevelamer is an ion-exchange resin that is commonly used as an alternative to calcium for phosphate binding. In addition to binding to phosphate, sevelamer has been shown to lower cholesterol, FGF-23, inflammatory markers, c-reactive protein and hemoglobin A1C and may improve endothelial function^[34,35]. In hemodialysis patients, sevelamer attenuates the progression of CAC and aortic calcification compared to calcium^[36-38] (Table 1). In two randomized controlled trials in incident hemodialysis patients, those who were treated with calcium had a greater risk of death compared to sevelamer^[2,39]. However, a randomized study in prevalent hemodialysis patients did not show survival benefit associated with sevelamer use^[40]. In non-dialysis CKD population, patients who were treated with sevelamer in order to keep serum phosphate within the normal range had better survival compared to those treated with calcium^[41]. In another small randomized study in moderate CKD patients that compared calcium, sevelamer and lanthanum versus placebo revealed an increase in arterial calcification in all groups, however, the degree was highest in the calcium group^[42]. It has been theorized that the use of phosphate binders in non-dialysis CKD may result in an increase in the availability of free calcium in the intestine. Similarly, when rosuvastatin, sevelamer and no drug were compared in a small randomized study in moderate CKD patients, a significant increase in CAC scores was observed in all three groups^[43]. Despite the possible survival benefit, the use of phosphate binders may not be beneficial in reducing calcification burden in moderate CKD population. In order to justify the use of phosphate binders in non-dialysis CKD patients, more studies are required to confirm the beneficial or harmful effects.

Lanthanum carbonate

Lanthanum is a rare earth element that is as effective as aluminum and better than sevelamer in binding phosphate^[44]. Long-term use of lanthanum in renal failure can result in an accumulation in various organs but without any obvious harmful effects^[45,46]. Similar to sevelamer, the use of lanthanum in moderate CKD can lower FGF-23 levels^[47]. In both uremic rats and dialysis patients, lanthanum attenuated the development of vascular calcifica-

tion^[48,49]. The data on patient-level outcomes are limited. A follow-up data on dialysis patients who were enrolled in the phase 3 study did not show survival benefit associated with lanthanum treatment. However, in a subgroup of patients > 65 years of age, those who received lanthanum carbonate appeared to have better survival compared to standard therapy^[50]. The efficacy of lanthanum depends largely on the pills being chewed thoroughly prior to swallowing. Recently the company has developed the oral powder form that may work better in patients with problems with mastication.

Combined calcium acetate-magnesium carbonate

Both intracellular and extracellular magnesium are vital in preventing inflammation and oxidative stress. Decreased magnesium concentration is associated with impaired endothelial function, vasospasm and atherogenesis^[51]. Increased severity of vascular calcification has been observed in hemodialysis and peritoneal dialysis patients with low normal magnesium levels^[52]. *In vitro* studies and *in vivo* study in rodents demonstrated that increasing magnesium concentrations were protective against vascular calcification through upregulation of anti-calcification proteins^[53-55]. In a study of 204 hemodialysis patients over a 24-wk follow-up, the European formulation of combined calcium-magnesium phosphate binder (calcium acetate 435 mg/magnesium carbonate 235 mg) was as efficacious as sevelamer in reducing serum phosphate without the side effect of increased serum ionized calcium. A small but significant increase in serum magnesium was observed. All patients were dialyzed against 0.5 mm magnesium dialysate and experienced no serious adverse events^[56]. FGF-23 levels also decreased in the magnesium group^[57]. Furthermore, a small observational study in 7 hemodialysis patients showed stabilization of CAC score after 18 mo of being on calcium-magnesium phosphate binder^[58].

Iron-based phosphate binders

Recently food and drug administration (FDA) approved iron-based phosphate binder in the United States is sucroferic oxyhydroxide. Another preparation of iron-based phosphate binder, ferric citrate, is currently under review by the FDA. These drugs are as efficacious as sevelamer in lowering serum phosphate^[59,60]. The use of iron-based phosphate binder is associated with an increase in serum ferritin and percent transferrin saturation leading to lesser requirement of intravenous iron and erythropoiesis-stimulating agents in dialysis patients^[61]. Iron deficiency can increase FGF-23 levels and therefore iron-based phosphate binders can lower FGF-23^[62]. In uremic rats, sucroferic oxyhydroxide prevented the development of vascular calcification^[63]. More information regarding iron-based phosphate binders should become available within the next year.

ACTIVE VITAMIN D

Active vitamin D are primarily used for the treatment of

Table 1 Studies related to therapies that may influence arterial calcification and patient outcomes

Ref.	Subjects	n	Study type	Intervention	Follow-up (mo)	Results
Braun <i>et al</i> ^[38]	HD	114	RCT	Sevelamer <i>vs</i> calcium	12	↓ CAC and AC
Chertow <i>et al</i> ^[36]	HD	200	RCT	Sevelamer <i>vs</i> calcium	12	↓ CAC
Kakuta <i>et al</i> ^[37]	HD	183	RCT	Sevelamer <i>vs</i> calcium	12	↓ CAC
Suki <i>et al</i> ^[40]	HD	2103	RCT	Sevelamer <i>vs</i> calcium	19	↔ mortality
Block <i>et al</i> ^[2]	Incident HD	127	RCT	Sevelamer <i>vs</i> calcium	44	↓ mortality
Di lorio <i>et al</i> ^[39]	Incident HD	466	RCT	Sevelamer <i>vs</i> calcium	24	↓ mortality
Block <i>et al</i> ^[42]	Non-dialysis CKD	148	RCT	Sevelamer, lanthanum, calcium <i>vs</i> placebo	9	↑ CAC and AC
Di lorio <i>et al</i> ^[41]	Non-dialysis CKD	212	RCT	Sevelamer <i>vs</i> calcium	24	↓ mortality
Lemos <i>et al</i> ^[43]	Non-dialysis CKD	38	RCT	Rosuvastatin, sevelamer <i>vs</i> no drug	24	↔ CAC
Toussaint <i>et al</i> ^[49]	HD	45	RCT	Lanthanum <i>vs</i> calcium	18	↓ AC
Wilson <i>et al</i> ^[50]	HD	1354	RCT	Lanthanum <i>vs</i> calcium	27	↔ mortality
Spiegel <i>et al</i> ^[58]	HD	7	Observational	Combined magnesium-calcium	18	↔ CAC
Kalantar-Zadeh <i>et al</i> ^[111]	HD	58058	Retrospective	Paricalcitol <i>vs</i> no drug	24	↓ mortality
Naves-Diaz <i>et al</i> ^[112]	HD	16004	Retrospective	Alfacalcidol or calcitriol <i>vs</i> no drug	16	↓ mortality
Shoji <i>et al</i> ^[113]	HD	242	Prospective	Alfacalcidol <i>vs</i> no drug	61	↓ CVD mortality
Tentori <i>et al</i> ^[114]	HD	38066	Retrospective	Active vitamin D <i>vs</i> no drug	60	↓ mortality
Melamed <i>et al</i> ^[115]	Incident HD and PD	1007	Prospective	Calcitriol <i>vs</i> no drug	30	↓ mortality
Teng <i>et al</i> ^[116]	Incident HD	51037	Retrospective	Active D <i>vs</i> no drug	24	↓ mortality
Tentori <i>et al</i> ^[117]	Incident HD	14967	Retrospective	Calcitriol <i>vs</i> paricalcitol <i>vs</i> doxercalciferol <i>vs</i> no drug	37	↓ mortality in all active D groups compared to no drug
Kovesdy <i>et al</i> ^[118]	Non-dialysis CKD	520	Retrospective	Calcitriol <i>vs</i> no drug	24	↓ mortality
Shoben <i>et al</i> ^[119]	Non-dialysis CKD	1418	Retrospective	Calcitriol <i>vs</i> no drug	24	↓ mortality
Sugiura <i>et al</i> ^[120]	Non-dialysis CKD	665	Retrospective	Alfacalcidol <i>vs</i> no drug	55	↓ CVD events and mortality
Thadhani <i>et al</i> ^[75]	Non-dialysis CKD	227	RCT	Paricalcitol <i>vs</i> placebo	48	↔ left ventricular mass index
Tamez <i>et al</i> ^[76]	Non-dialysis CKD	196	RCT	Paricalcitol <i>vs</i> placebo	48	↓ left atrial volume index
Raggi <i>et al</i> ^[80]	HD	360	RCT	Cinacalcet + active D <i>vs</i> active D	12	↓ CAC and aortic valve calcification
Chertow <i>et al</i> ^[83]	HD	3883	RCT	Cinacalcet <i>vs</i> placebo	21	↔ CVD events or mortality
Hashiba <i>et al</i> ^[88]	HD	18	RCT	Etidronate <i>vs</i> no drug	6	↓ AC
Nitta <i>et al</i> ^[87]	HD	35	Observational	Etidronate	12	↓ CAC
Kawahara <i>et al</i> ^[91]	GP	108	RCT	Atorvastatin <i>vs</i> etidronate <i>vs</i> both	12	↓ thoracic and abdominal aortic plaques in combined therapy
Adirekhiat <i>et al</i> ^[33]	HD	32	Prospective	STS <i>vs</i> no drug	9	↓ CAC
Mathews <i>et al</i> ^[98]	HD	22	Observational	STS	5	↓ CAC

n: Number of patients; HD: Hemodialysis; PD: Peritoneal dialysis; CKD: Chronic kidney disease; RCT: Randomized controlled trial; CAC: Coronary calcification; AC: Aortic calcification; CVD: Cardiovascular disease; STS: Sodium thiosulfate.

hyperparathyroidism in CKD. In addition to lowering PTH, active vitamin D also stimulates calcium and phosphate absorption in the gastrointestinal tract and therefore can result in worsening hypercalcemia and hyperphosphatemia. Active vitamin D also reduces proteinuria, augments the response to erythropoietin and suppresses renin-angiotensin system^[64-66]. The parent drug of active vitamin D is calcitriol or 1, 25-dihydroxyvitamin D₃. The closely related analogs to calcitriol are alfacalcidol (1- α hydroxyvitamin D₃) and doxercalciferol (1- α hydroxyvitamin D₂). Both require 25-hydroxylation process in the liver prior to becoming active forms. Similar to the parent compound, alfacalcidol and doxercalciferol can precipitate hypercalcemia and hyperphosphatemia especially if given in high doses. Paricalcitol or 19-Nor-1-25-dihydroxyvitamin D₂ was developed specifically for

the treatment of hyperparathyroidism in CKD. Paricalcitol appears to act preferentially in the parathyroid glands and less so in the gastrointestinal tract^[67]. In rodents with uremia, administration of calcitriol and doxercalciferol resulted in an increase in aortic calcification, whereas paricalcitol did not^[68]. However when testing different doses of calcitriol and paricalcitol, both active vitamin D in high doses induced a similar degree of aortic calcification. Interestingly, in this study, lower doses of both calcitriol and paricalcitol seemed to be protective against vascular calcification^[69]. The calcemic and phosphatemic effects of all forms of active vitamin D have been confirmed in a recent randomized crossover trial in hemodialysis patients that showed similar incidences of hyperphosphatemia and hypercalcemia among patients who received alfacalcidol or paricalcitol^[70]. The increase in calcium and

phosphate load as a result of active vitamin D induced calcium and phosphate absorption is likely responsible for the development of vascular calcification. On the other hand, the direct effect of vitamin D on vascular wall appears to be positive. Active vitamin D can stimulate klotho and osteopontin expression in the arterial wall. Both of which help prevent vascular calcification^[71]. This finding can probably explain the protective effect of low dose active vitamin D on vascular calcification. The development of vascular calcification associated with the use of active vitamin D is the result of systemic accumulation of calcium and phosphate rather than the local effect on arterial wall^[72]. Therefore, low doses of active vitamin D that do not augment calcium and phosphate load may actually be protective against vascular calcification^[23,69].

As for the beneficial effect of vitamin D on renin-angiotensin system, a study in rats with renal failure demonstrated that active vitamin D treatment could prevent left ventricular hypertrophy and myocardial fibrosis^[73]. In an observational study in hemodialysis patients, treatment of hyperparathyroidism with intravenous calcitriol led to a decline in renin, angiotensin II and atrial natriuretic peptide levels associated with a decrease in left ventricular hypertrophy^[74]. However, a recent randomized study in moderate CKD patients revealed only a non-significant trend toward a decrease in left ventricular mass index in the group of patients that received paricalcitol^[75]. Nevertheless, subsequent analysis did demonstrate a significant decrease in left atrial volume index^[76]. The lack of clear benefit of active vitamin D on cardiac hypertrophy may be related to the increase in FGF-23 in response to active vitamin D treatment. As for survival benefit of active vitamin D therapy in hemodialysis patients, several retrospective and observational studies have revealed a decrease in all-cause and cardiovascular mortality among patients who received active vitamin D regardless of PTH levels^[77]. Details of these studies can be found in Table 1. The benefit seemed to be more pronounced in the low-dose range and among patients who received paricalcitol. At the present time, there is no published prospective randomized study that evaluates the effect of active vitamin D on survival in CKD population.

CALCIMIMETIC

Calcimimetic allosterically activates calcium-sensing receptors, thus can suppress PTH secretion without elevating serum calcium. Calcimimetic is used as an add-on to active vitamin D and phosphate binder in the treatment of hyperparathyroidism in CKD^[78]. Currently, cinacalcet is the only calcimimetic drug available for this purpose. In nephrectomized rats, adding cinacalcet to active vitamin D helped decrease the severity of vascular calcification associated with high dose vitamin D treatment^[79]. In a randomized study in 360 hemodialysis patients, the rate of progression of CAC and aortic valve calcification was reduced when cinacalcet was added to low dose active vitamin D compared to larger and varying doses of ac-

tive vitamin D therapy alone^[80,81]. Cinacalcet therapy also decreases FGF-23 levels^[82]. However, significant benefits in terms of overall survival or cardiovascular events were not observed in a large randomized controlled trial in 3883 hemodialysis patients after 5 years of follow-up^[83].

BISPHOSPHONATES

Bisphosphonates are synthetic analogs of inorganic pyrophosphate that have the ability to suppress bone resorption and therefore are commonly used in the treatment and prevention of osteoporosis in general population. The other important property of inorganic pyrophosphate is inhibition of calcium and phosphate crystal deposition in the bone matrix. Oral etidronate and intravenous pamidronate have been utilized in the treatment of calcific uremic arteriopathy (CUA), a condition of wide spread small-vessel calcification that results in progressive cutaneous ulcer due to ischemia^[84,85]. In uremic rats, daily pamidronate or etidronate therapies prevented aortic calcification^[86]. In hemodialysis patients, oral and parenteral etidronate have been shown to delay the progression of CAC and aortic calcification^[87,88]. However, this anti-calcification effect was not observed with the newer generation bisphosphonates including alendronate and ibandronate^[89,90]. A recent randomized study in general population with hypercholesterolemia revealed the combined regimen of daily atorvastatin and etidronate reduced atherosclerotic plaque burden in thoracic and abdominal aorta^[91]. It was suggested that etidronate was responsible for the regression of calcified plaques in abdominal aorta while atorvastatin attenuating the non-calcified plaques in thoracic aorta. Worsening adynamic bone disease with the use of bisphosphonates in the setting of CKD is of concern, thus recent Kidney Disease Improving Global Outcomes recommendation advised against prescribing bisphosphonates in patients with an eGFR < 30 mL/min per 1.73 m²^[92].

SODIUM THIOSULFATE

Sodium thiosulfate (STS) is a reducing, chelating and anti-oxidant agent that is useful as an antidote in cyanide poisoning. STS also has the ability to chelate calcium in precipitated minerals forming calcium thiosulfate that is more soluble than calcium oxalate and calcium phosphate. Thus its use has been expanded in conditions with increased calcification burden such as nephrolithiasis, metastatic calcification, tumoral calcinosis and CUA^[93-96]. In a large observational study in 172 hemodialysis patients with CUA, intravenous STS therapy resulted in clinical improvement in most patients^[96]. In uremic rats, parenteral administration of STS has been shown to prevent the development of vascular calcification^[97]. Twice weekly intravenous STS therapy in hemodialysis patients was able to delay the rate of progression of CAC after six months compared to the non-treatment group but with a significant decline in hip bone mineral density in one study^[33,98]. Long-term intravenous or intraperitoneal

STS therapy in dialysis patients are well tolerated with minimal side effects^[33,96,99]. The mechanism by which STS reduces calcification burden is poorly understood. It has been suggested that mechanisms other than calcium chelation are responsible for the decreased calcification burden^[94,100].

VITAMIN K

There are two types of naturally occurring vitamin K: vitamin K1 (phylloquinone) found mostly in green leafy vegetables and vegetable oils and vitamin K2 (menaquinone) found in animals, bacteria, and fermented food such as cheese and natto. Five to twenty five percent of ingested vitamin K1 can be converted to vitamin K2 in the body. Colonic bacteria can synthesize vitamin K2 and antibiotics that interfere with the growth of these colonic flora impair vitamin K2 production^[101]. Vitamin K is required as a co-factor in the process of gamma-carboxylation of several extracellular matrix proteins turning inactive uncarboxylated proteins into active carboxylated forms. Prothrombin, coagulation factors 7, 9 and 10 require vitamin K1 for their carboxylation processes; whereas, osteocalcin and matrix gla-protein require vitamin K2^[102]. Osteocalcin is important in bone mineralization; therefore, menaquinone is used in the treatment of osteoporosis. Matrix gla-protein is a calcification inhibitor that plays important role in the prevention of arterial calcification. Warfarin, an antagonist to vitamin K, not only inhibits coagulation but long-term use can also promote arterial calcification^[103]. Vitamin K deficiency is common in end-stage renal disease patients and the accumulation of inactive form of matrix gla-protein is associated with an increase in the severity of arterial calcification and mortality^[104,105]. High menaquinone intake is also associated with reduced CAC and coronary heart disease in general population^[106,107]. Vitamin K1 or K2 supplementation especially in high doses can significantly decrease the amount of inactive matrix gla-protein in hemodialysis patients^[108]. In CKD rats treated with warfarin, high dietary vitamin K1 can blunt the development of vascular calcification^[109]. The favorable impact of vitamin K1 on vascular calcification is likely depending on the conversion of vitamin K1 to vitamin K2 in the body. A prospective randomized controlled trial to evaluate the effect vitamin K1 supplementation on the progression of CAC (VitaVasK trial) in hemodialysis patients is currently ongoing^[110].

In conclusion, the currently available treatment options for arterial calcification in CKD include non-calcium containing phosphate binders, low doses of active vitamin D, calcimimetic agent and perhaps bisphosphonates, vitamin K and STS. Preliminary data on bisphosphonates, vitamin K and STS are encouraging but larger studies on efficacy and outcomes are needed.

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WJC 6th Anniversary Special Issues (1): Hypertension

Management of hypertension in primary aldosteronism

Anna Aronova, Thomas J Fahey III, Rasa Zarnegar

Anna Aronova, Thomas J Fahey III, Rasa Zarnegar, Department of Surgery, Weill Cornell Medical College/New York Presbyterian Hospital, New York, NY 10021, United States

Author contributions: Aronova A, Fahey III TJ and Zarnegar R contributed equally to the conception and acquisition of data, drafting and revision the manuscript for intellectual content and approving the final version for publication.

Correspondence to: Rasa Zarnegar, MD, Department of Surgery, Weill Cornell Medical College/New York Presbyterian Hospital, 585 East 68th Street, A1027, New York, NY 10021, United States. raz2002@med.cornell.edu

Telephone: +1-212-7465130 Fax: +1-212-7469948

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Abstract

Hypertension causes significant morbidity and mortality worldwide, owing to its deleterious effects on the cardiovascular and renal systems. Primary hyperaldosteronism (PA) is the most common cause of reversible hypertension, affecting 5%-18% of adults with hypertension. PA is estimated to result from bilateral adrenal hyperplasia in two-thirds of patients, and from unilateral aldosterone-secreting adenoma in approximately one-third. Suspected cases are initially screened by measurement of the plasma aldosterone-renin-ratio, and may be confirmed by additional noninvasive tests. Localization of aldosterone hypersecretion is then determined by computed tomography imaging, and in selective cases with adrenal vein sampling. Solitary adenomas are managed by laparoscopic or robotic resection, while bilateral hyperplasia is treated with mineralocorticoid antagonists. Biochemical cure following adrenalectomy occurs in 99% of patients, and hemodynamic improvement is seen in over 90%, prompting a reduction in quantity of anti-hypertensive medications in most patients. End-organ damage secondary to hypertension and excess aldosterone is significantly improved by both surgical and medical treatment, as

manifested by decreased left ventricular hypertrophy, arterial stiffness, and proteinuria, highlighting the importance of proper diagnosis and treatment of primary hyperaldosteronism. Although numerous independent predictors of resolution of hypertension after adrenalectomy for unilateral adenomas have been described, the Aldosteronoma Resolution Score is a validated multifactorial model convenient for use in daily clinical practice.

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Key words: Primary hyperaldosteronism; Hypertension; Adrenalectomy; Aldosteronoma; Treatment

Core tip: Primary hyperaldosteronism is the most common reversible form of secondary hypertension. After appropriate diagnosis and localization studies, adrenalectomy is the procedure of choice for unilateral aldosterone-secreting adenomas, while medical therapy is best for bilateral adrenal hyperplasia. Surgical resection improves or cures biochemical and hemodynamic perturbations in most patients, and halts or reverses many of the deleterious effects of hyperaldosteronism. Predicting which patients will benefit most from adrenalectomy is aided by the Aldosteronoma Resolution Score.

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INTRODUCTION

Hypertension is one of the most prominent risk factors for morbidity and mortality worldwide, accounting for 45% of deaths due to heart disease and 51% due to stroke^[1,2]. In the United States alone, 69 million adults

(29%) have hypertension, in whom it is significantly associated with myocardial infarction, cerebrovascular accidents, heart failure and renal disease^[3,4]. Given the large impact on global health, controlling hypertension is of utmost importance. Significant efforts have been made to characterize potentially curable, or secondary, types of hypertension such as renovascular hypertension, pheochromocytoma, Cushing's syndrome and primary hyperaldosteronism.

Primary hyperaldosteronism (PA) is the leading cause of secondary hypertension, and can be identified in 5% to 18% of hypertensive patients^[5,6]. First described by Conn in 1955 in a patient presenting with resistant hypertension and hypokalemia who was found to have an aldosterone-secreting adrenal adenoma^[7], PA can present in a myriad of clinical scenarios. Most recent epidemiologic studies have shown that approximately 60% of patients are found to have bilateral idiopathic hyperplasia, also known as idiopathic hyperaldosteronism (IHA), while 30% present with unilateral aldosterone-producing adenomas (APA)^[8]. One to two percent of patients present with primary or unilateral adrenal hyperplasia (UAH), 1% with aldosterone-secreting adrenocortical carcinoma, 1% with familial hyperaldosteronism, and 1% with ectopic aldosterone-producing adenoma or carcinoma^[6,9,10].

Classically, excessive aldosterone secretion not only results in difficult to manage hypertension in the majority of patients, but also produces biochemical effects of hypokalemia in 10%-30% of patients^[11]. More recent data, however, suggest that most patients with PA are actually normokalemic^[6,11,12]. In addition, aldosterone hypersecretion has been linked to significant and potentially reversible end-organ damage, particularly in the cardiovascular and renal systems^[13]. For instance, Tanabe *et al.*^[14] demonstrated that patients with PA have more pronounced cardiac hypertrophy compared to patients with essential or other secondary causes of hypertension. Fortunately, timely correction of aldosterone levels can prevent or reverse some of these effects^[15]. This review will describe the current methods of diagnosis and management of primary hyperaldosteronism, with a particular focus on the systemic effects of adrenalectomy as well as the predictors of resolution of hypertension after surgery.

DIAGNOSIS

Patients with hypertension and hypokalemia, regardless of suspected cause (diuretics, incidentaloma), and patients with medically-resistant hypertension, should be considered for screening for primary hyperaldosteronism^[16]. Initial evaluation of patients involves biochemical testing with plasma aldosterone (ng/dL) to renin (ng/mL per hour) ratio (ARR). This test identifies excessive aldosterone secretion with simultaneous suppression of plasma renin activity. Although ARR is regarded as the ideal screening tool for PA, there exists some controversy regarding the clinical conditions under which the ARR is obtained, as well as the test's diagnostic accuracy. Certain drugs, including beta-blockers, angiotensin-converting enzyme inhibi-

tors (ACE-I), selective-serotonin reuptake inhibitors and oral contraceptives, have been shown to affect the results of the test^[17,18]. Ideal testing conditions involve discontinuation of such medications two weeks prior^[10,17,18]. However, in a recent study, Fisher *et al.*^[19] showed that doing so is impractical, and most patients are unable to be taken off their anti-hypertensive medications without the need for substitution by other agents to adequately control blood pressure or serious side effects such as hospitalization. Others suggest that only use of spironolactone will absolutely interfere with the interpretation of this ratio^[16]. In addition, there is some disagreement regarding the requirement of a minimum plasma aldosterone level and the critical ARR cutoff for diagnosis. Most authors recommend an ARR of 20-40, and researchers found that ARR of at least 35 has 100% sensitivity and 92.3% specificity in diagnosing primary hyperaldosteronism^[17,20,21]. Furthermore, biochemical testing should be done in the morning, in a seated position after an initial two-hour ambulatory period^[18]. False negative and positive results can occur, as affected by age, smoking, medications, posture, and renal function, so it is generally advisable to repeat biochemical testing in patients with high pretest probability of PA, typically four weeks later^[18].

Patients with suspected primary aldosteronism identified by screening ARR may undergo confirmatory testing or go on to localization studies. Confirmatory testing includes: the oral sodium loading test, the saline infusion test, the fludrocortisone suppression test, and the captopril challenge test^[22]. Time, cost, patient compliance, and certain physiologic parameters need to be considered in choosing the specific confirmatory test. For instance, in patients with severe hypertension, cardiac or renal insufficiency, clinicians should avoid the oral sodium loading test and the saline infusion tests. In general, such additional testing often proves burdensome and in 30%-50% of cases does not prove to be abnormal in patients with high ARR suggestive of PA^[10,22,23]. Currently, there is lack of evidence encouraging the use of any one of these tests as a gold standard and many physicians, including those in our own practice, no longer recommend confirmatory testing.

LOCALIZATION

The etiology of aldosterone hypersecretion is established by imaging and adrenal vein sampling (AVS). The distinction between unilateral APA from bilateral hyperplasia is a key factor in determining the appropriate management. APAs are best managed by surgical resection, whereas the treatment for IHA is medical therapy. Current high-resolution computed tomography (CT) imaging has enhanced the classification of subtypes of hyperaldosteronism and the ability to identify APAs. The sensitivity and specificity of adrenal imaging with 1.25-3 mm cuts for APA is 78% and 75%, respectively^[22,24]. Findings on adrenal CT include normal-appearing adrenals, unilateral macroadenomas (greater than 1 cm), unilateral microadenomas (less than 1 cm), bilateral micro- or macroadeno-

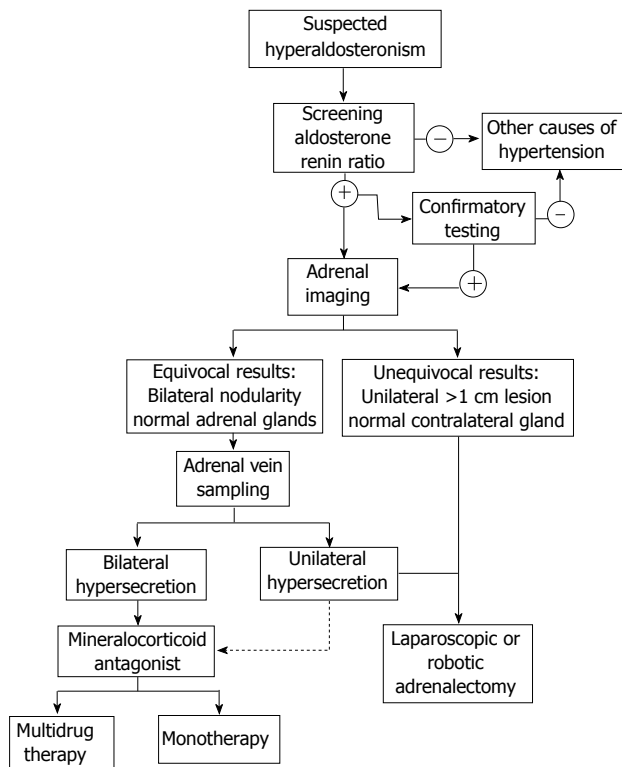


Figure 1 Treatment algorithm.

mas, and minimal unilateral adrenal limb thickening^[22]. Imaging in IHA can reveal normal-appearing adrenal glands or show nodular changes. As a result, radiologists can misread APAs as IHA, whereas microadenomas can be incorrectly labeled as areas of hyperplasia^[22]. Several studies have shown that CT alone may lead to misdiagnosis in PA. In a systematic review, Kempers *et al.*^[25] found that 37.8% of patients who showed lateralization on CT/magnetic resonance imaging (MRI) had conflicting results on AVS. If imaging alone was used for localization, 14.6% of patients would have undergone inappropriate adrenalectomy, while 19.1% would have been inappropriately excluded from surgery. Furthermore, in 3.9% of patients, CT/MRI lateralized to the opposite side. These considerations have prompted many to regard AVS as a gold standard for lateralization. However, mandatory use remains a contentious topic. The United States Endocrine Society and Japan Endocrine Society guidelines recommend that AVS be performed in all patients who have diagnosed PA and are considering surgical resection^[22,25,26]. However, the Adrenal Vein Sampling International Study showed that AVS is utilized routinely in only a few centers worldwide^[27]. AVS requires highly skilled radiologists for successful cannulation of both adrenal veins and the procedure is not without complications. AVS is unsuccessful in up to 20% due to failure to cannulate the right adrenal vein, and even in experienced centers, the complication rate averages 0.5%–2.5%^[24,25,28,29].

Despite recommendations from the endocrine societies, several groups continue to advocate for selective use. Zarnegar *et al.*^[30] and Tan *et al.*^[31] both demonstrated the

effectiveness of AVS in cases of equivocal findings on initial imaging studies. Specifically, Zarnegar *et al.*^[30] compared outcomes after adrenalectomy for patients with > 1 cm adenomas with normal contralateral adrenal glands on CT to those who required AVS and CT (< 1 cm). They found similar outcomes in both groups as measured by biochemical and hemodynamic resolution, advocating for selective use of AVS for patients with smaller tumors or indeterminate imaging findings. A recently-issued consensus statement recommends certain patients with PA do not necessarily require AVS, including: patients who are < 40 years old with marked PA and clear unilateral adrenal adenoma and normal contralateral gland on imaging; patients who are not surgical candidates due to unacceptably high operative risk; patients with suspected adrenocortical carcinoma; or patients who have proven familial hyperaldosteronism^[32].

MANAGEMENT

Treatment of PA is aimed at prevention of morbidity and mortality associated with hypertension, hypokalemia and direct aldosterone-associated organ damage. Once the cause of hyperaldosteronism is established, the proper management strategy can be instituted (Figure 1). Adrenalectomy is the procedure of choice for documented unilateral secretion of aldosterone (APA or UAH), while medical therapy is warranted for bilateral aldosterone hypersecretion as with IHA and bilateral APA, or for patients who refuse surgery or are poor surgical candidates.

Medical management involves antagonism of the mineralocorticoid (MR) receptor with spironolactone or eplerenone. Spironolactone has been utilized for over four decades as a first-line agent at doses ranging 25–400 mg/d^[22,33]. Hypokalemia typically resolves immediately, but blood pressure reduction may take several months to occur^[6]. Anti-androgen side effects such as gynecomastia and dysmenorrhea can result from spironolactone due to cross-antagonism of the sex-steroid receptors, usually in a dose-dependent fashion^[34,35]. Eplerenone is more specific for the aldosterone receptor and therefore causes fewer undesired side effects. It is, however, less potent^[36]. A recent randomized trial comparing the two therapies showed that spironolactone from 75 to 225 mg/d was more efficacious than eplerenone at 100–300 mg/d for hypertension control^[36]. In addition, since spironolactone is cheaper and more widely available, clinicians should weigh these factors when recommending the appropriate agent for medical management of PA^[10,36]. It is noteworthy that hypervolemia can be prohibitive in using MR antagonists as sole agents for PA, and in approximately 50% of patients, a second agent such as a low-dose thiazide diuretic can help achieve adequate blood pressure control^[37]. Other agents including sodium channel blockers (amiloride, triamterene), calcium channel blockers, ACE-I, and angiotensin-receptor blockers (ARB) have also been employed as secondary agents in PA, with variable effects on blood pressure and plasma aldosterone levels^[37,38].

Adrenalectomy is the preferred treatment strategy for

patients with demonstrable unilateral hypersecretion of aldosterone. The standard approach employed by most centers is lateral transperitoneal laparoscopic adrenalectomy as first described in 1992 by Gagner *et al*^[39]. However, some surgeons prefer a posterior retroperitoneoscopic approach or robotic-assisted surgery. Proponents of the retroperitoneoscopic approach recommend this technique for smaller tumors (< 6 cm), prior abdominal surgery and lower body mass index (BMI)^[40-42]. Several recent meta-analyses comparing transabdominal to retroperitoneal laparoscopic adrenalectomy found no significant differences between the two approaches^[43,44]. Additionally, Brandao *et al*^[45] systematically reviewed robotic-assisted adrenalectomy and found that it is equally safe and may even result in less blood loss and shorter hospital stay, compared to laparoscopic approaches.

OUTCOMES

Aldosterone hypersecretion causes hypertension and biochemical abnormalities with potassium hemostasis by activation of the renin-angiotensin-aldosterone-system (RAAS). It has been shown that abnormal activation of the RAAS correlates directly with end-organ damage in the cardiovascular and renal systems and it is well-documented that blockade of the angiotensin-II arm by ACE-I or ARB provides significant cardiovascular protection^[13]. Pathophysiologically, aldosterone works to increase sodium absorption in the kidneys, leading to increased intravascular volume and thereby increased blood pressure. Cardiovascular damage occurs from increased left ventricular mass and hypertrophy as well as aldosterone-driven fibrosis and collagen production in the interventricular septum. Furthermore, perivascular inflammation, vascular remodeling in the heart and kidney, and direct damage to the nephron anatomy and physiology, are thought to contribute to sustained deleterious end-organ effects from aldosterone excess that may occur independent of hypertension^[46-48]. In fact, compared to patients with essential hypertension, patients with primary hyperaldosteronism are at increased risk for these adverse effects, which are significantly reduced by surgical or medical management^[49-51]. Milliez *et al*^[52] demonstrated in a retrospective study a markedly increased incidence of stroke (12.9% *vs* 3.4%), non-fatal MI (4.0% *vs* 0.6%), and atrial fibrillation (7.3% *vs* 0.6%) in patients with PA compared to those with essential hypertension. There was no difference in the PA subtype. Additionally, Ribstein *et al*^[53] reported significant decrease in proteinuria in patients with PA with treatment of aldosterone excess by adrenalectomy or spironolactone compared to control essential hypertension patients.

The treatment of aldosterone hypersecretion either by medical or surgical means is very effective. Nearly 100% of patients will experience a biochemical cure with normalization of hypokalemia and aldosterone levels^[54,55]. These effects follow surgery relatively quickly. It is recommended that potassium supplements and MR antagonists should be discontinued on post-operative day 1, and anti-

hypertensive medications reduced simultaneously. Patients are also instructed to eat a diet generous in salt for the first month after surgery to account for a suppressed contralateral adrenal gland^[56]. Interestingly, a minority of people can develop prolonged zona glomerulosa insufficiency causing hyperkalemia after adrenalectomy. Reported by Fischer *et al*^[57], this outcome had an incidence of 5% of adrenalectomized PA patients in their cohort and required long-term fludrocortisone treatment post-operatively.

Resolution of hypertension in primary hyperaldosteronism is etiology-specific. For cases not appropriate for surgical resection, blood pressure control is best achieved by mineralocorticoid antagonists, as previously discussed. Conversely, for localized APAs adrenalectomy results in improvement in blood pressure control in over 90% of patients, and complete resolution, as defined by BP < 140/90 mmHg without the need for antihypertensive medications, in 30%-60%^[6,58]. Patients that are not cured generally experience lower mean blood pressures and take fewer antihypertensive medications after surgery^[59]. Persistent hypertension after adrenalectomy may result from misdiagnosis of unilateral aldosterone hypersecretion, or more likely, coexistent essential hypertension with underlying end organ damage. Chronic aldosterone excess has been shown to increase arterial stiffness, and may contribute to enduring hypertension in these patients^[60]. Blood pressure typically normalizes or shows maximal improvement in one to six months after adrenalectomy, though it can continue to decrease for up to one year following surgery^[60].

Multiple studies have looked at outcomes of adrenalectomy for APA to characterize predictive factors for resolution of hypertension. Factors that have been correlated with favorable results include younger age, female sex, lower BMI, fewer pre-operative antihypertensive medications, shorter duration of hypertension preoperatively, fewer first-degree family members with hypertension, better renal function as evidenced by higher glomerular filtration rate, lower creatinine and less proteinuria, lower serum aldosterone and higher urine aldosterone, histopathologic features, and smaller tumor size^[58,61-64]. Recently, in a large series, Zhang *et al*^[65] showed by multivariate regression that shorter duration of hypertension and lower serum aldosterone level were predictive of resolution of hypertension after adrenalectomy. Furthermore, several studies have linked the *TT* genotype of *CYP11B2* gene encoding aldosterone synthase to successful outcomes after adrenalectomy for PA^[66-68].

To better predict which of these features result in resolution of hypertension after adrenalectomy in patients with APA, Zarnegar *et al*^[55] proposed the Aldosteronoma Resolution Score (ARS) which takes into account four readily available pre-operative clinical parameters including BMI ≤ 25 kg/m², female sex, duration of preoperative hypertension ≤ 6 years, and number of preoperative antihypertensive medications ≤ 2. Each parameter receives a score of 1, with the exception of number of preoperative medications, which is scored by 2 points due to its relative significance in the prediction model. A score

of 0-1 predicts a low likelihood of resolution, while patients with ARS 4-5 have a high likelihood of resolution of hypertension after adrenalectomy. In the study, 27.6% of patients with ARS 0-1 were cured, whereas 75% with ARS 4-5 had complete resolution of hypertension. Using an external cohort, the authors also demonstrated external validity of the model. Utsumi *et al.*^[61] further validated the accuracy of the ARS model using a Japanese population, confirming the utility of the ARS as a clinical tool for counseling patients on expected surgical outcomes.

While surgery abolishes the source of excess aldosterone secretion and significantly improves or resolves biochemical disturbances and blood pressure control, the long-lasting effects of exposure on the vasculature, heart, brain and kidney have yet to be completely delineated^[63]. Nonetheless, several studies have shown that the progression of at least some of these effects are slowed or even reversed by adrenalectomy. Strauch *et al.*^[60] showed that resection of APA reduced arterial stiffness parameters compared to medical management. Rossi *et al.*^[15] showed regression of left ventricular hypertrophy in patients with primary hyperaldosteronism after appropriate medical or surgical intervention compared to optimally treated patients with primary hypertension, while Lin *et al.*^[69] showed adrenalectomy reversed myocardial fibrosis in these patients. Renal function has also been shown to improve after resection with resolution of microalbuminuria in APA patients compared to those with essential hypertension owing to the resolution of relative glomerular hyperfiltration in PA from the volume-expanding and hypertensive effects of the hormone^[50,70].

CONCLUSION

Primary hyperaldosteronism is a common and treatable cause of secondary hypertension. Aldosterone excess has been linked to systemic disturbances in the cardiovascular, renal, and vascular systems, in addition to causing hypokalemia and hypertension. Multiple studies have shown worse morbidity with higher rates of myocardial infarction, stroke and renal dysfunction compared to patients with essential hypertension. Depending on the subtype, medical or surgical treatment is effective at halting or even reversing some, if not all, of these effects. Diagnosis and subtype differentiation relies on ARR, possible confirmatory testing, and localization studies with CT and adrenal venous sampling. Unilateral adrenalectomy for patients with APA successfully reverses biochemical disturbances, resolves or significantly improves hypertension, and halts progression of systemic perturbations. Though a variety of parameters have been found to be associated with resolution of hypertension after resection of APA, the ARS is currently the most accurate prediction model for resolution. Adrenalectomy for APA is a safe procedure that should be performed for appropriate candidates to improve long-term outcomes.

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WJC 6th Anniversary Special Issues (1): Hypertension

Anti-hypertensive drugs in children and adolescents

Patricia Y Chu, Michael J Campbell, Stephen G Miller, Kevin D Hill

Patricia Y Chu, Kevin D Hill, Duke Clinical Research Institute, Durham, NC 27715, United States

Michael J Campbell, Stephen G Miller, Kevin D Hill, Division of Pediatric Cardiology, Department of Pediatrics, Duke University Medical Center, Durham, NC 27715, United States

Author contributions: Chu PY, Campbell MJ, Miller SG and Hill KD contributed to the manuscript literature review, data compilation and writing.

Correspondence to: Kevin D Hill, MD, MSCI, Assistant Professor of Pediatrics in the Division of Pediatric Cardiology, Division of Pediatrics, Duke University Medical Center, Duke Clinical Research Institute, 2400 Pratt Street, Room 7582, Box 3850 Durham, NC 27705, United States. kevin.hill@duke.edu

Telephone: +1-919-6684686 Fax: +1-919-6687058

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Abstract

Worldwide the prevalence of essential hypertension in children and adolescents continues to increase. Traditionally providers have used "off-label" drugs to treat pediatric hypertension, meaning that rigorous clinical trials of these drugs have not been specifically performed in pediatric patient populations. Consequently providers have extrapolated dosing, safety and efficacy from trials in adults. This practice is sub-optimal as children demonstrate unique differences in drug metabolism and response. Use of unstudied or understudied drugs increases risk of adverse events and/or can lead to sub-optimal efficacy. Recognizing these concerns, regulatory agencies have created financial incentives for industry to conduct pediatric clinical trials. These incentives, coupled with the emerging pediatric hypertension epidemic, have spurred over 30 clinical trials of anti-hypertensive drugs over the past 15 years and have resulted in labeling of 10 new drugs by the United States Food and Drug Administration for treatment of hypertension in children and adolescents. Unfortunately the financial incentive structures focus on newer drugs and drug classes. Consequently there is now a relative dearth of trial data for older but sometimes commonly

prescribed pediatric antihypertensive drugs. This article reviews recent pediatric antihypertensive drug trials with a focus on trial design and endpoints, drug dosing, safety, efficacy and specific drug indications. We also review the available data and experience for some of the more commonly prescribed, but less well studied "older" pediatric antihypertensive drugs.

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Key words: Hypertension; Children; Clinical trials; Dosing; Safety

Core tip: This review focuses on the major clinical trials of anti-hypertensive drugs that have been completed over the past 15 years in response to regulatory initiatives by the United States Food and Drug Administration and the European Medicines Agency. These trials have changed the landscape of anti-hypertensive drug management in children.

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INTRODUCTION

Nations throughout the developed world are facing an emerging epidemic of pediatric hypertension that has paralleled an increasing prevalence of childhood obesity^[1-5]. In recent cross-sectional studies, greater than one out of every seven United States children and adolescents demonstrate prehypertension with over 3% meeting diagnostic criteria for hypertension^[6]. Prevalence trends are similar in population-based assessments in numerous other nations^[7-11]. Elevated blood pressure during childhood and adolescence is associated with end organ damage^[12,13], most commonly left ventricular hypertrophy, and

is predictive of hypertension in early adulthood^[5,14,15].

With increasing prevalence of pediatric hypertension, there is a need for data supporting safety and efficacy of antihypertensive drugs. While a wide variety of antihypertensive drugs have been studied in clinical trials in adults, traditionally there has been a paucity of evidence to support safety and efficacy of antihypertensive drugs in children and adolescents. Consequently, providers were forced to use drugs “off-label”, extrapolating dosing and efficacy from adult data^[16]. This practice is sub-optimal as children demonstrate unique physiology and pathology, and off-label drug use risks inadequate disease treatment and/or safety events. Furthermore most drugs designed for use in adults do not have pediatric specific tablets or formulations, which can complicate dosing. Recognizing these concerns, regulatory agencies in both the United States and Europe have passed recent regulatory initiatives aimed at stimulating pediatric clinical trials^[17,18]. These initiatives have been very successful and over the preceding 15 years, more than 20 clinical trials of antihypertensive agents have been completed in children leading to approval of 10 drugs by the United States Food and Drug Administration (FDA) for treatment of hypertension in children and/or adolescents (Figure 1).

This review summarizes the available data and experience supporting the use of antihypertensive drugs in children and adolescents diagnosed with essential hypertension with a particular focus on recent pediatric clinical trials. Angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor blockers, calcium channel blockers, beta blockers and diuretics will be covered with a critical appraisal of available clinical trial data supporting dosing, efficacy, safety, and treatment in specific patient populations. Approval of drugs for pediatric use by the United States FDA will be used as a meaningful benchmark of adequate drug study, reflecting the stringent standards required for FDA approval.

IDENTIFICATION OF CLINICAL TRIAL DATA

To identify anti-hypertensive drug trials in children and adolescents, we used four principle sources: the United States FDA website (<http://www.accessdata.fda.gov/>), the FDA approved drug label, the European Medicines Agency (EMA) website (<http://www.ema.europa.eu/>) and PubMed. The FDA website and drug label include detailed information summarizing clinical trials completed in response to an FDA issued written request (a requirement for trials completed for drug labeling) including trial design, drug dosing, efficacy and safety data. Similarly the EMA publishes the results of reviews conducted for EMA pediatric drug approval. We also reviewed publications cited on PubMed for relevant clinical trials. Publications were identified following a PubMed search restricted to children and adolescents ≤ 18 years and using MeSH terms “Hypertension” and “clinical trial”.

ANGIOTENSIN CONVERTING ENZYME INHIBITORS

ACE inhibitors target the renin-angiotensin-aldosterone-system (RAAS). ACE converts angiotensin I to angiotensin II (Ang II), a peptide that causes vasoconstriction and stimulates aldosterone production, itself a potent vasoconstrictor. ACE inhibitors lower blood pressure by decreasing Ang II and mitigating its downstream effects. In adults, ACE inhibitors are commonly used antihypertensives and have the additional benefit of reducing cardiovascular and renal events^[19]. In pediatric populations, ACE inhibitors are the most commonly prescribed antihypertensive for both primary and secondary hypertension^[20,21]. ACE inhibitors have anti-proteinuric effects and are particularly beneficial in children with chronic kidney disease^[22-24] (Table 1). However, similar to adult trials, pediatric trials provide evidence that some ACE inhibitors may be less efficacious in blacks^[25-27]. In adult anti-hypertensive trials, side effects associated with use of ACE inhibitors include hyperkalemia, chronic cough and angioedema. In pediatric trials there have been no reports of angioedema and there are fewer reports of cough in pediatric compared to adult trials. However, many of the pediatric trials have been of shorter duration^[28]. ACE inhibitors are teratogenic and should be discontinued as soon as pregnancy is detected. ACE inhibitors approved for treatment of pediatric hypertension by the FDA include enalapril, fosinopril, benazepril and lisinopril. Table 2 summarizes the FDA label dosing recommendations that resulted from review of the various pediatric clinical trials of these ACE inhibitors.

Enalapril^[29]

Enalapril was the first ACE inhibitor approved by the United States FDA for pediatric hypertension following completion of the required clinical trials in 2002 (Figure 1). Compared to placebo, children treated with moderate or high doses (2.5 or 20 mg for children < 50 kg and 5 mg or 40 mg for children > 50 kg) demonstrated significantly lowered diastolic blood pressure (DBP) and systolic blood pressure (SBP). However, the low dose group (0.625 mg/1.25 mg) did not demonstrate lowering of DBP or SBP. There was no significant difference in antihypertensive effects across race, age, sex or Tanner stage. Enalapril was well tolerated and safe in the four-week trial. The most common side effects were dizziness (3.6%) and headache (1.8%), and there was only one drug discontinuation ($< 1\%$) due to adverse events. The enalapril FDA label is unique in that the drug has a pediatric indication for all young children with the only exception being neonates.

Fosinopril^[25,30]

Fosinopril was approved for treatment of pediatric hypertension by the United States FDA after the trials (including a 52-wk open label safety assessment) were completed in 2003 (Figure 1). In the clinical trials, all three

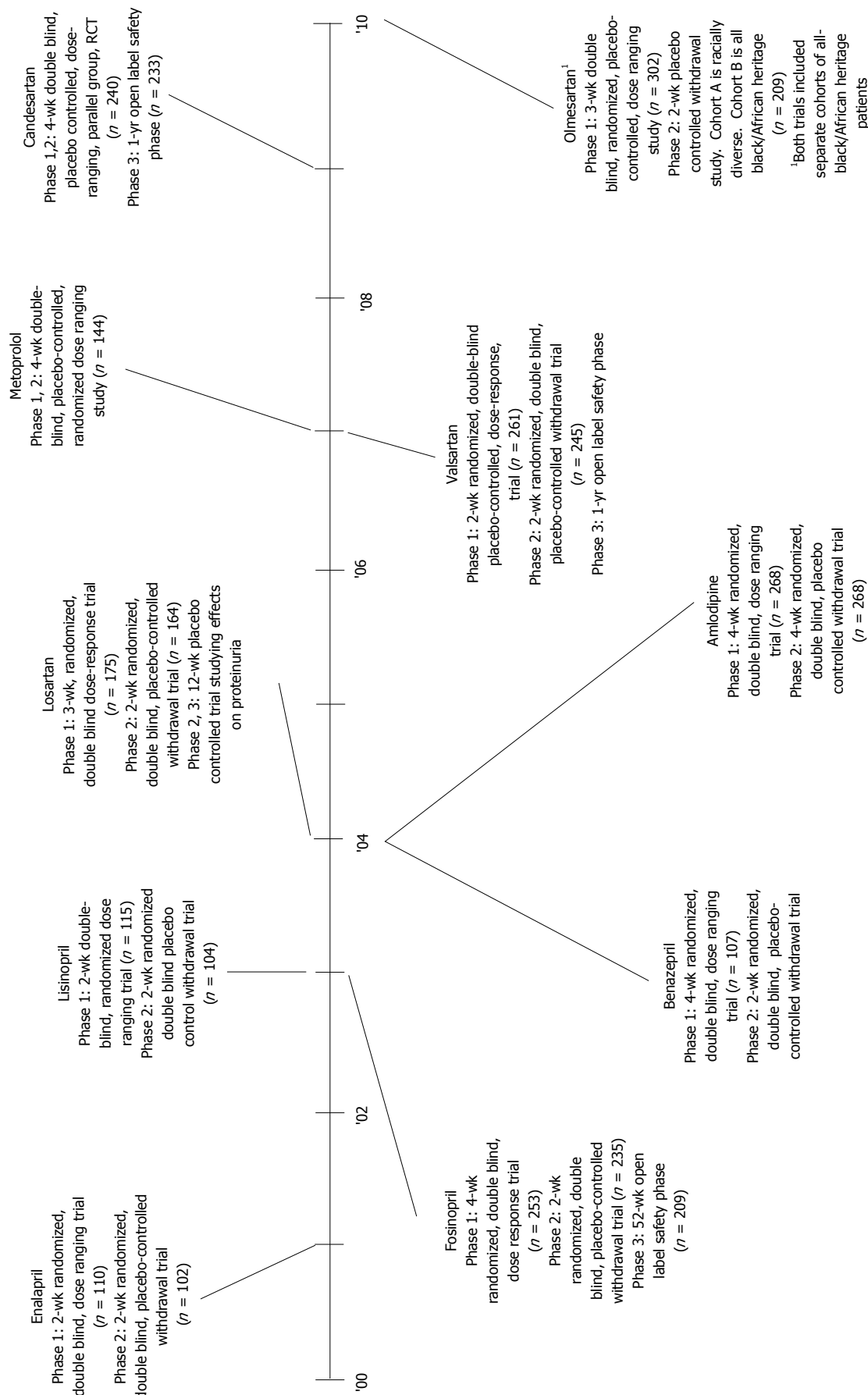


Figure 1 Timeline for completion of trials that have resulted in United States Food and Drug Administration labeling for treatment of hypertension in children and adolescents.

Table 1 Anti-hypertensive class effects

Drug class	Special indications	Precautions	Contraindications	Common adverse events
Angiotensin converting enzyme inhibitors	Proteinuria Chronic kidney disease	Less efficacious in blacks Risk of angioedema, increase risk of hyperkalemia Decreased glomerular filtration rate	Prior history of angioedema with use of ACE inhibitor Discontinue if pregnant: Pregnancy class C in 1 st trimester, pregnancy class D in 2 nd and 3 rd trimester	Headache Dizziness Abdominal pain Nausea Cough
Angiotensin receptor blockers	Proteinuria	Less efficacious in blacks Increase risk of hyperkalemia Decreased GFR	Discontinue if pregnant: Pregnancy class C in 1 st trimester, pregnancy class D in 2 nd and 3 rd trimester	Headache Dizziness Cough
Calcium channel blockers	None	Drug interactions with compounds that change cytochrome P450s metabolism (<i>i.e.</i> : Azole antifungals, grapefruit juice, anti-seizure medications)	Pregnancy class C	Headache Peripheral edema Fatigue Dizziness Abdominal pain Epistaxis
Beta blockers	None	Increased risk of bronchoconstriction in asthma	Severe bradycardia Heart block greater than first degree Cardiogenic shock Decompensated cardiac failure	Headache Cough Nasopharyngitis Fatigue Diarrhea Dizziness

Pregnancy class C: Animal reproduction studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks; Pregnancy class D: There is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks; ACE: Angiotensin converting enzyme.

Table 2 Anti-hypertensive drugs that have been studied in pediatric clinical trials for United States Food and Drug Administration labeling

Drug class	Drug	Starting dose	Max dose	Frequency	Suspension formulation	Pediatric indication
Angiotensin converting enzyme inhibitor	Enalapril	0.08 mg/kg (up to 5 mg)	0.58 mg/kg or 40 mg	Daily	Yes	All except neonates
	Fosinopril	0.1 mg/kg (5-10 mg)	0.6 mg/kg or 40 mg	Daily	No	Children > 50 kg
Angiotensin receptor blocker	Lisinopril	0.07 mg/kg (up to 5 mg)	0.6 mg/kg or 40 mg	Daily	Yes	> 6 yr
	Benazepril	0.2 mg/kg (up to 10 mg)	0.6 mg/kg or 40 mg	Daily	Yes	> 6 yr
	Losartan	0.7 mg/kg (up to 50 mg)	1.4 mg/kg or 100 mg	Daily	Yes	> 6 yr
	Valsartan	1.3 mg/kg (up to 40 mg)	2.7 mg/kg or 160 mg	Daily	Yes	> 6 yr
	Candesartan	1-6 yr: 0.2 mg/kg 6-17 yr, < 50 kg: 4 mg 6-17 yr, > 50 kg: 8 mg	1-6 yr: 0.4 mg/kg 6-17 yr, < 50 kg: 16 mg 6-17 yr, > 50 kg: 32 mg	Daily or divided dose	Yes	> 1 yr
	Olmesartan	20 to < 35 kg: 10 mg ≥ 35 kg: 20 mg	20 to < 35 kg: 20 mg ≥ 35 kg: 40 mg	Daily	Yes	> 6 yr
	Irbesartan	No FDA pediatric indication (efficacy not demonstrated)				
Beta blocker	Metoprolol XL	1.0 mg/kg (< 50 mg)	2 mg/kg up to 200 mg	Daily	No	> 6 yr
	Bisoprolol	No FDA pediatric indication (efficacy not demonstrated)				
Calcium channel blocker	Amlodipine	2.5 mg	0.3 mg/kg or 10 mg	Daily	No	> 6 yr
	Felodipine	No FDA pediatric indication (efficacy not demonstrated)				
Diuretic	Eplerenone	No FDA pediatric indication (efficacy not demonstrated)				

FDA: Food and Drug Administration.

dose levels (0.1, 0.3 and 0.6 mg/kg) of fosinopril were equally effective at reducing SBP and DBP with no dose response in the overall cohort. It remains unclear whether the lack of dose response was attributable to: (1) the dose levels being too high; (2) an overly narrow dose range; or (3) true absence of a dose response. Further analysis showed that fosinopril was effective at reducing SBP in a dose responsive manner in black children however, blacks required a higher dose per body weight to achieve

adequate control^[25]. Fosinopril was well tolerated with no serious adverse events in the 52-mo open label extension study. Discontinuation of fosinopril secondary to adverse events during the dose ranging and withdrawal phase was rare (1.6%). In the open label extension phase 83% successfully reached target BP with headache (20.1%), nasopharyngitis (9.6%), cough (9.1%), pharyngitis (8.6%), and abdominal pain (6.2%) being the most common adverse events.

Table 3 Other commonly used “off-label” antihypertensive drugs¹

Drug class	Drug	Starting dose	Max dose	Frequency
Angiotensin converting enzyme inhibitor	Captopril	0.3-0.5 mg/kg per dose	6 mg/kg up to 450 mg/d	Two to three times daily
Beta blocker	Atenolol	0.5 mg/kg per day	2 mg/kg per day up to 100 mg	Once to twice daily
	Propranolol	1 mg/kg per day	16 mg/kg per day up to 640 mg	Two to four times daily
Calcium channel blocker	Extended release nifedipine	0.25 mg/kg per day	3 mg/kg per day up 120 mg/kg per day	Once to twice daily
Diuretic	Furosemide	0.5 mg/kg per dose	6 mg/kg per dose	Twice to three times daily
	Hydrochlorothiazide	0.5-1 mg/kg	3 mg/kg up to 50 mg	Daily

¹These drugs have not been well studied in pediatric clinical trials and dosing/safety/efficacy are largely extrapolated from trials in adults.

Lisinopril^[31]

Lisinopril was approved for pediatric hypertension by the United States FDA in 2003. In the pivotal trial (Figure 1), lisinopril demonstrated a dose response reduction in SBP and DBP that was consistent across age groups, tanner stages, and ethnicity. Lisinopril was safe and well tolerated in the four-wk trial with no serious adverse events and few discontinuations (< 1%). The most common adverse events were headache (3.5%), dizziness from hypotension (1.7%), and abdominal pain (1.7%).

Benazepril^[32]

Pediatric trials for benazepril have not been published in the literature, but the United States FDA approved it for pediatric hypertension in 2004 and the trials are summarized on the FDA label (Figure 1). Benazepril significantly lowered SBP but did not exhibit a dose response. Benazepril was well tolerated. The FDA label does not report if any patients discontinued the trial due to drug related adverse events.

Captopril

Captopril is not approved for treatment of hypertension in children and adolescents, as it is an off-patent agent with no financial incentive for industry to sponsor clinical trials. Because captopril was one of the earliest ACE inhibitors approved for use in adults, there is a substantial body of clinical experience in children and adolescents and several trials have demonstrated clinical efficacy^[33,34]. However, a major disadvantage of captopril is the need for frequent dosing (typically three times per day) (Table 3).

ANGIOTENSIN RECEPTOR BLOCKERS

Angiotensin receptor blockers (ARBs) target the Angiotensin II type 1 receptors located on the heart, kidney, blood vessels, and adrenal glands. By blocking the final step of the RAAS, ARBs inhibit vasoconstriction and lower blood pressure^[35]. Similar to ACE inhibitors, ARBs are particularly beneficial in reducing left ventricular hypertrophy in adults with heart failure. In adults and children, ARBs are effective at reducing proteinuria secondary to diabetes and may be particularly useful in patients with chronic kidney disease^[36-38] (Table 1). However, ARBs are generally less efficacious in African Americans^[26,39-42]. Adults who experience cough and can-

not tolerate ACE inhibitors often take ARBs as an alternative^[43]. ARBs approved for the treatment of pediatric hypertension include losartan, valsartan, candesartan, and olmesartan. Table 2 summarizes the FDA label dosing recommendations that resulted from review of the various pediatric clinical trials of these ARBs. Children tolerated ARBs well, and the side effects most frequently experienced were headache and dizziness.

Losartan^[38,44]

Losartan was the first ARB approved for pediatric hypertension by the United States FDA in 2004 following completion of the required clinical trials (Figure 1). Losartan demonstrated a dose response reduction in SBP and DBP with efficacy demonstrated for the moderate and high dose groups (2.5 or 25 mg for children < 50 kg and 5.0 or 50 mg for children ≥ 50 kg) but no significant difference in BP between the low dose Losartan or placebo group. There were too few non-white patients to evaluate race related differences in dose response. Losartan was well tolerated with few discontinuations due to adverse events (< 1%).

Losartan was also studied in a clinical trial focused on reduction of proteinuria in hypertensive ($n = 60$) and normotensive ($n = 246$) children with chronic kidney disease^[38]. Losartan reduced proteinuria by 35.9% (95%CI: 27.6%-43.1%) and was superior to both placebo (normotensive cohort) and amlodipine (hypertensive cohort). Additionally, Losartan reduced SBP and DBP in both cohorts and was superior to amlodipine, although authors postulated that a lack of change in BP in children on amlodipine was due to titration effect. There were no serious adverse events in this trial and 0.7% of subjects discontinued losartan due to adverse events.

Valsartan^[45]

Valsartan was approved for pediatric use by the United States FDA in 2007. The Valsartan pediatric clinical trials are summarized in Figure 1. Valsartan demonstrated a dose response reduction in SBP and DBP but no statistically significant difference in blood pressure between the low or medium-dose groups (10, 20 mg for children < 35 kg and 20, 40 mg for children ≥ 35 kg). Valsartan's antihypertensive effects were observed across all subgroups including sex, age, tanner stage and race (black and non-black). During the dose response and withdrawal phase

of the study, there were no serious adverse events and few subjects (1.6%) discontinued therapy due to adverse events. Headache (11.6%) and dizziness (2.7%) were the most commonly reported adverse events in the dose response phase. In the 52-wk open label trial, 3.6% of subjects discontinued valsartan due to adverse events. Gastroenteritis (< 1%) and hyperkalemia (< 1%) were the only adverse events considered to be drug-related.

Candesartan^[46]

Candesartan was approved for pediatric use by the United States FDA in 2009. Pediatric clinical trials are summarized in Figure 1. In the dose ranging study, Candesartan demonstrated a significant decrease in SBP and DBP compared to placebo at all dose levels but not a dose response. The lack of dose response was attributed to a narrow dose range^[46,47]. In the extension study, the 1-year response rate (SBP < 95%) was 52%. Black children had a lesser reduction in SBP and DBP and a lower response rate compared to white children (response rate in black *vs* white 43 *vs* 61%). Drug discontinuation due to adverse events was rare (1% in dose ranging study and 2.1% in open label study) and there were no serious adverse events.

Olmesartan^[48]

Olmesartan was approved for pediatric hypertension by the United States FDA in 2010. In clinical trials (Figure 1) olmesartan demonstrated a dose response reduction in SBP and DBP, but the BP reduction was smaller in blacks. Olmesartan was well tolerated and drug discontinuation due to adverse events was rare (< 1%) with no serious adverse events. The most commonly experienced side effects in the 6-wk period were headache (1.7%) and dizziness (1.3%).

Irbesartan^[49,50]

Irbesartan was not approved for pediatric hypertension due to lack of efficacy. The irbesartan pediatric trials (Figure 1) failed to demonstrate a dose response and although subjects demonstrated statistically significant increases in blood pressure following drug withdrawal, the effect size (+ 2.3 mg Hg increase in SBP) was small and was not felt to be clinically meaningful. Adverse events were more frequent than in other ARB trials and 2.5% discontinued study drug. There was also one case of erythema multiforme possibly related to irbesartan use.

CALCIUM CHANNEL BLOCKERS

Calcium channel blockers (CCBs) encompass a diverse group of agents with different targets and functions. Second and third generation dihydropyridine CCBs, such as felodipine and amlodipine, are highly selective for vascular smooth muscle and are commonly prescribed for pediatric hypertension^[20,21,51]. They target L type (long acting) voltage sensitive calcium channels and inhibit further influx of calcium into already depolarized smooth muscle cells, thereby inhibiting actin-myosin activation

and muscle contraction^[51]. Unlike ACE inhibitors and ARBs, dihydropyridine CCBs do not demonstrate any anti-proteinuric effects in adults^[52-54]; however, other studies have shown renoprotective effects in renal transplant patients^[55].

Side effects associated with CCBs include gingival hyperplasia and lower extremity edema. Other side effects such as flushing and headache are more commonly associated with immediate release preparations used for acute hypertension. Dihydropyridine CCBs are metabolized/excreted by the liver and dosing can be affected by drugs or compounds that alter CYP metabolism (*e.g.*, Azole antifungals, grapefruit juice)^[51]. Pediatric trials have been performed for the CCBs amlodipine and felodipine and FDA dosing recommendations from these trials are summarized in Table 2. Only amlodipine is approved for treatment of pediatric hypertension as felodipine did not demonstrate efficacy.

Amlodipine^[56]

Amlodipine was approved for pediatric hypertension by the United States FDA in 2004. It is the most commonly prescribed CCB for pediatric hypertension^[21]. In pediatric trials (Figure 1), amlodipine demonstrated a dose response reduction in SBP and DBP. SBP reduction was slightly greater in females compared to males; otherwise, SBP reduction across race, age, and etiology of HTN did not differ significantly. Amlodipine was generally well tolerated with few discontinuations due to adverse events (2.2%). Reasons for discontinuation included worsening hypertension (1.1%), facial edema (< 1%), edema of the fingers with rash (< 1%), and premature ventricular contractions (< 1%). Peripheral edema, an adverse event commonly seen in adults, was reported in 3.8% of children in dose ranging phase and 2.3% of children in placebo withdrawal phase.

Felodipine ER^[57]

Felodipine is a long acting calcium channel blocker that has not been approved for pediatric HTN due to lack of efficacy. The felodipine pediatric trial included a three-wk dose response trial (*n* = 128) in children with primary hypertension and a 14-wk open label extension period to assess safety. Felodipine was well tolerated (0.8% discontinued due to adverse event) and there were no serious adverse events.

Nifedipine

Nifedipine is a calcium channel blocking agent that was previously frequently prescribed to children and adolescents but was off patent and did not qualify for financial incentives and therefore has not been specifically studied for FDA labeling. Data are lacking on efficacy of short acting nifedipine and concerns have been raised about the dosing formulations which can lead to significant blood pressure fluctuations^[34,58]. Sustained release nifedipine perhaps has more utility but also has not been formally studied in children and adolescents and therefore must be used "off-label"^[34] (Table 3).

BETA BLOCKERS

Beta blockers have been used for over 40 years and are recommended for hypertension treatment in adults with coronary artery disease, heart failure, post-myocardial infarction, and diabetes because of their beneficial cardiac effects^[59]. Beta blockers lower blood pressure by antagonizing the beta 1 adrenergic receptor located on the myocardium to reduce heart rate and decrease contractility. However, beta blockers may also act on beta 2 adrenergic receptors on the smooth muscle of vasculature and the bronchi, increasing peripheral resistance and risk of bronchospasm^[60]. Second generation beta blockers such as metoprolol, bisoprolol, and atenolol are relatively more selective for beta 1 receptors compared to first generation non-selective beta blockers, but at high doses, they may act on beta 2 receptors. Compared to other antihypertensives, first and second generation beta blockers are associated with a higher rate of insulin resistance and new onset diabetes^[60-64]. The newest class of beta blockers including carvedilol and nebivolol are vasodilatory and do not appear to have negative effects on metabolic profile^[60-63].

Bisoprolol and extended release (XR) metoprolol have been studied in pediatric populations for the treatment of hypertension and their FDA dosing recommendations are summarized in Table 2. In both trials, children with asthma were excluded because of the drugs' potential broncho-constrictive effects. Bisoprolol did not demonstrate efficacy and, as a result, extended release metoprolol is the only FDA approved beta blocker for pediatric hypertension. Carvedilol has also been studied in pediatric populations but for the treatment of heart failure^[65,66]. Efficacy was not demonstrated and, although indicated for treatment of hypertension in adults, carvedilol has never been studied for this indication in children or adolescents. Nonetheless there are data to support dosing of a pediatric formulation^[65]. In all pediatric trials of beta blockers drug-related serious adverse events were rare.

Metoprolol^[67]

Metoprolol was approved for pediatric hypertension by the United States FDA in 2007. In clinical trials (Figure 1) metoprolol significantly reduced SBP compared to placebo, but with no dose-response effect. Only high doses of XR metoprolol (2 mg/kg) demonstrated significant reductions in DBP compared to placebo. Authors postulated that the lack of dose response reduction in SBP may have been due to a flattening of the dose response curve or a limitation of the study design. At the end of the dose ranging study, the response rate for metoprolol was 46% (95%CI: 37%-55%). Metoprolol's anti-hypertensive effects were independent of age, Tanner stage, and race. Authors note that overweight patients (BMI > 95%) tended to have less pronounced SBP reductions. Metoprolol was safe and well tolerated with a maximum decrease in heart rate of only 6.5 beats per minute.

Drug discontinuation was rare in all trial phases (0.7% in the dose response phase and 5.9% in the open label trial). The most commonly reported adverse events were headache (30%), upper respiratory tract infection (20%), cough (19%), nasopharyngitis (13%), pharyngolaryngeal pain (12%), fatigue (9%), diarrhea (7%), and dizziness (6%).

Bisoprolol fumarate/hydrochlorothiazide^[68]

Bisoprolol fumarate/hydrochlorothiazide (HCT) (B/HT) is a combination hypertensive that failed to gain United States FDA approval for pediatric hypertension due to lack of efficacy. In a placebo controlled dose ranging pediatric trial ($n = 94$), the percentage of patients in the B/HT group that achieved blood pressure control (SBP and DBP < 90th%) was not significantly different from placebo (45% for B/HT, 34% for placebo). Discontinuation of B/HT due to adverse events was rare (1.6%) and overall fewer adverse events were reported for the B/HT group compared to placebo.

Propranolol and atenolol

As some of the oldest beta blockers, propranolol and atenolol fall into the category of off-patent drugs that have not qualified for financial incentives and no large pediatric trials have been performed. As a result, propranolol and atenolol are not labeled for treatment of hypertension in children and adolescents. Most pediatric studies of these beta blockers have been in small case series or for other non-hypertensive indications such as arrhythmias, syncope, hypertrophic cardiac cardiomyopathy, portal hypertension. In these studies, propranolol and atenolol have been effective with acceptable tolerability^[34]. Due to the lack of pediatric data, dosing, safety, and efficacy have been extrapolated from adult trials (Table 3).

DIURETICS

Most diuretics were off-patent before the implementation in Europe and the United States of financial incentives to conduct pediatric trials. Because off-patent drugs do not qualify for the financial incentives, diuretics represent the class of anti-hypertensive drugs with the least available pediatric clinical trial data. The only diuretic to be tested in a pediatric trial is eplerenone, but it was not approved due to lack of efficacy. Because other diuretics are often used as first line treatment in adults, they will be discussed briefly. Table 3 summarizes generally recognized (albeit not well studied) dosing recommendations for diuretics and select other commonly used antihypertensive drugs that are off-patent and thus have not been studied in clinical trials for FDA or EMA labeling.

Overall, diuretics are a diverse class of drugs that contain some of the oldest and most commonly prescribed agents for adult hypertension^[59,69,70]. They can be broadly divided into three categories, thiazide diuretics, loop diuretics, and potassium sparing diuretics. All three classes target different parts of the nephron to decrease sodium

and water reabsorption, thereby creating a natriuretic effect that decreases extracellular volume and reduces blood pressure.

Potassium sparing diuretics

Potassium sparing diuretics inhibit reabsorption of sodium in the collecting duct and can be further divided into two groups, pteridine analogs and aldosterone antagonists. Pteridine diuretics inhibit epithelial sodium channels (ENaC) and aldosterone antagonist down regulate the Na/K pump and (ENaC) on the collecting duct. Potassium sparing diuretics are often used in conjunction with other potassium losing diuretics to maintain serum potassium levels in a normal range^[71,72]. Eplerenone is the only diuretic to be studied for FDA labeling but was not approved. In adults, eplerenone is sometimes preferred over spironolactone because it more selectively binds to aldosterone receptors and does not have unwanted progestational and anti-androgenic effects^[72].

Eplerenone^[73,74]

Eplerenone is a selective aldosterone antagonist that was not approved for pediatric hypertension by the United States FDA due to lack of efficacy. The pediatric trial consisted of a 6-wk dose ranging study ($n = 304$) and a 4-wk dose withdrawal study ($n = 277$). Children on concomitant therapy with a potent CYP3A4 inhibitor (clarithromycin, ketoconazole), potassium supplement, or potassium level > 5.5 mEq/L were excluded and eplerenone is considered contraindicated under such circumstances. In children ages 4 to 17 years old, eplerenone did not demonstrate a dose-response effect and reduced SBP was only seen for the high dose level (50 mg twice a day for children > 20 kg). There was no significant difference in DBP compared to the placebo group. Eplerenone was well tolerated with few serious adverse events (2.6%) or discontinuations in the ten-wk trial ($< 1\%$).

Thiazide diuretics

Thiazide diuretics, such as hydrochlorothiazide and chlorthalidone, are first line agents for uncomplicated adult hypertension and are commonly combined with beta blockers, loop diuretics, and ACE inhibitors in multi-drug regimens and in fixed-dose combination formulations^[59,75,76]. They are preferred because of their efficacy and superiority in preventing cardiovascular disease compared to other classes of antihypertensives^[77]. Thiazides block sodium-chloride co-transporters on the distal convoluted tubule to decrease sodium reabsorption; however, these effects are acute. The exact mechanism by which thiazides reduce peripheral resistance and chronically lower blood pressure is unknown^[71,78]. Thiazides are contraindicated in patients with sulfa allergies. Side effects in adults include hypokalemia, hypercalcemia, orthostatic hypotension, worsening of gout (due hyperuricemia), and a worsened metabolic profile (increased rates of new onset diabetes, increase in low density lipoprotein (LDL) cholesterol triglycerides, and glucose)^[64,71,78].

Loop diuretics

Loop diuretics (furosemide, bumetanide, torsemide, ethacrynic acid) are most commonly prescribed in combination with thiazide diuretics for reducing fluid volume in edematous disorders or patients with renal failure^[71,79]. There is no data supporting the efficacy of loop diuretics alone to reduce blood pressure. When prescribed alone, loop diuretics lower blood pressure acutely, but not chronically because the activated RAAS will compensate for the lost fluid volume. Loop diuretics inhibit the sodium/potassium/chloride transporter (Na-K-2Cl transporter) on the thick ascending loop of Henle to decrease the osmotic gradient producing a potent natriuretic effect. All loop diuretics, other than ethacrynic acid are contraindicated in patients with sulfa allergies. Side effects in adults associated with loop diuretics include hyponatremia, hypokalemia, hypocalcemia, hypomagnesemia, and worsening of metabolic profile (increased cholesterol, LDL, and triglycerides)^[71,80].

CONCLUSION

Regulatory initiatives in the United States and Europe over the last one and a half decades have stimulated numerous clinical trials of antihypertensive agents in children. The result has been an increase in the number of United States FDA approved drugs for treatment of pediatric hypertension from zero in 2000 to 11 at present (including esmolol approved for intravenous administration). This is very encouraging with the only caveat that most of the medications studied in pediatric trials belong to newer classes of drugs. There remains a relative dearth of clinical trial data regarding the safety and efficacy of older, commonly used antihypertensive drugs (*e.g.*, diuretics) in children. Nonetheless pediatric providers can now rely on clinical trial data to guide many treatment decisions in children and adolescents with hypertension. FDA labeled antihypertensive drugs have all been safe, efficacious and well tolerated. No deaths and only rare serious adverse events have been reported in clinical trials, albeit most have been of shorter duration. Furthermore, these clinical trials have highlighted the differences between drug safety and efficacy in children versus adults. Many of the approved drugs have demonstrated differences in dosing when compared to adult recommendations and several drugs approved for use in adult patient populations (irbesartan, bisoprolol fumarate/HCTZ, felodipine and eplerenone) have not demonstrated efficacy in pediatric hypertension trials. These data highlight that pediatric drug dosing, safety and efficacy cannot simply be extrapolated from adult clinical trials.

As the prevalence of childhood obesity and hypertension continue to rise, it is critical that providers familiarize themselves with these clinical trial data to guide appropriate treatment. Lifestyle changes should continue to form the mainstay of pediatric hypertension therapy; however the importance of medical therapy is increasingly recognized as a means to prevent end-organ damage and hope-

fully limit the long-term cardiovascular risk associated with hypertension.

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WJC 6th Anniversary Special Issues (1): Hypertension

Alcohol-induced hypertension: Mechanism and prevention

Kazim Husain, Rais A Ansari, Leon Ferder

Kazim Husain, Leon Ferder, Department of Physiology, Pharmacology and Toxicology, Ponce School of Medicine and Health Sciences, Ponce, PR 00732, United States

Rais A Ansari, Department of Pharmaceutical Sciences, College of Pharmacy, Nova Southeastern University, Fort Lauderdale, FL 33314, United States

Author contributions: Husain K designed, performed the research and wrote the review article; Ansari RA contributed the new tools for writing the review article; Ferder L contributed the guidance and suggestions for writing the review article.

Correspondence to: Kazim Husain, PhD, DABT, Professor, Department of Physiology, Pharmacology and Toxicology, Ponce School of Medicine and Health Sciences, PO Box 7004, Ponce, PR 00732, United States. khusain@psm.edu

Telephone: +1-787-8402575 Fax: +1-787-8413736

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Abstract

Epidemiological, preclinical and clinical studies established the association between high alcohol consumption and hypertension. However the mechanism through which alcohol raises blood pressure remains elusive. Several possible mechanisms have been proposed such as an imbalance of the central nervous system, impairment of the baroreceptors, enhanced sympathetic activity, stimulation of the renin-angiotensin-aldosterone system, increased cortisol levels, increased vascular reactivity due to increase in intracellular calcium levels, stimulation of the endothelium to release vasoconstrictors and loss of relaxation due to inflammation and oxidative injury of the endothelium leading to inhibition of endothelium-dependent nitric oxide production. Loss of relaxation due to inflammation and oxidative injury of the endothelium by angiotensin II leading to inhibition of endothelium-dependent nitric oxide production is the major contributors of the alcohol-induced hypertension. For the prevention of alcohol-induced hypertension is to reduce the amount of alcohol intake. Physical conditioning/exercise training

is one of the most important strategies to prevent/treat chronic alcohol-induced hypertension on physiological basis. The efficacious pharmacologic treatment includes the angiotensin-converting enzyme (ACE) inhibitors or angiotensin II type 1 receptor blockers (ARBs) which have antioxidant activity and calcium channel blockers. The most effective prevention and treatment of alcohol-induced hypertension is physical exercise and the use of ACE inhibitors or ARBs in the clinic

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Key words: Alcohol; Hypertension; Mechanisms; Prevention/treatment; Vascular endothelium

Core tip: This is a comprehensive review of the current mechanisms of alcohol-induced hypertension and strategies for prevention and treatment of alcohol-related hypertension. This updated review will be imperative to basic scientist in the area of cardiovascular physiology/pharmacology and clinicians in the academic, industry as well as clinics and hospitals.

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INTRODUCTION

Alcohol (ethyl alcohol or ethanol, C₂H₅OH) from fermented grain, fruit juice and honey have been used for thousands of years. Fermented beverages existed and alcoholic drinks used in early Egyptian civilization, in China around 7000 BC, in India, between 3000 and 2000 BC, in Babylon as early as 2700 BC, in Greece, and in South America^[1]. In the sixteenth century, alcohol (called “spirits”) was used largely for medicinal purposes^[2]. At the beginning and mid of the eighteenth century, spirits was

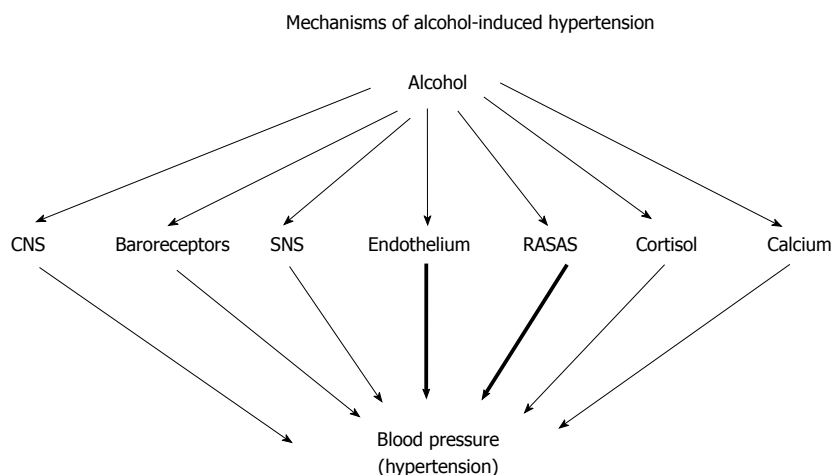


Figure 1 Mechanisms of alcohol-induced hypertension. CNS: Central nervous system; SNS: Sympathetic nervous system; RASAS: Renin-angiotensin system and aldosterone system.

used heavily in Britain. The nineteenth century brought a change in attitudes and the temperance movement began promoting the moderate use of alcohol. In 1920 the United States passed a law prohibiting the manufacture, sale, import and export of intoxicating liquors. Current research suggests that the moderate consumption of alcohol is beneficial to the cardiovascular system and lowers the blood pressure^[3-5]. A preclinical study also showed a decrease in systolic blood pressure in rats fed ethanol (1.0 g/kg) for 12 wk^[6]. Moderate drinking is generally considered to be: Two drinks a day for men younger than age 65, one drink a day for men age 65 and older and one drink a day for women of any age. A drink is 12 ounces (355 milliliters) of beer, 5 ounces (148 milliliters) of wine or 1.5 ounces (44 milliliters) of 80-proof distilled spirits. Low to moderate drinking has been shown to reduce the incidence of coronary heart disease^[3-5] and to increase longevity. It has clearly been a major analgesic, and one widely available to people in pain^[1,2,7].

Today, alcoholic beverages are consumed regularly by most of the human societies in the world. However its abuse is a major public health problem in the world. In United States alcohol abuse affects more than 20 million individuals leading to loss of 100000 lives annually^[8,9]. Chronic high dose ethanol consumption most commonly causes hepatic, gastrointestinal, nervous and cardiovascular injuries leading to physiological dysfunctions^[10]. A cause and effect relationship between regular alcohol consumption and blood pressure elevation (hypertension) was first suggested in 1915 by Lian *et al*^[11]. Recent epidemiological and clinical studies have demonstrated that chronic ethanol consumption (more than three drinks per day, 30 g ethanol) is associated with an increased incidence of hypertension and an increased risk of cardiovascular diseases^[12-17]. The magnitude of the increase in blood pressure in heavy drinkers averages about 5 to 10 mmHg, with systolic increases nearly always greater than diastolic increases^[18]. Similar changes in blood pressure were also reported in preclinical studies^[19-22]. In the Framingham cohort^[23,24], there was an increase of 7 mmHg in mean arterial pressure when heavy alcohol users were compared with all others. In some epidemiological studies a linear dose-response relationship has

been established, sometimes starting with a consumption threshold of 3 drinks per day (30 g of ethanol)^[25-33]. In others, the relationship has been nonlinear, especially in women, and some authors have speculated that ingestion of smaller quantities of alcohol may reduce blood pressure^[34-38]. Only a few studies have addressed the relationship between alcohol and hypertension in the elderly, and most of them have shown a strong association between hypertension prevalence and alcohol intake^[39,40]. However preclinical studies have also shown a linear relationship between blood pressure and ingestion of alcohol^[6]. The molecular mechanisms and possible mediators through which alcohol causes vascular injury and raises blood pressure remain elusive. This review focuses the mechanisms implicated with alcohol-induced hypertension and the strategies to control, prevent or to treat alcohol-induced elevation of blood pressure.

MECHANISMS OF ALCOHOL-INDUCED HYPERTENSION

There are several possible mechanisms through which alcohol can raise the blood pressure as shown in Figure 1.

Central nervous system in alcohol-induced hypertension

The World hypertension League speculated that the relatively greater effect alcohol on systolic blood pressure compared with diastolic blood pressure may indicate an imbalance between central nervous system factors influencing cardiac output and the peripheral vascular effects of alcohol^[41,42]. There is increasing evidence that alcohol initiates central as well as peripheral reactions which in a synergistic manner have a hypertensive action. In addition, alcohol induces an increased sympathetic outflow, most probably linked to secretion of corticotropin-releasing hormone^[43]. Some investigators have suggested that the association between alcohol and hypertension is related to the temporal sequence of alcohol use and blood pressure measurement^[24,44]. Since many community programs require an overnight or twelve-hour fasting period, alcohol withdrawal, albeit subclinical, may be oc-

curing. Similarly, patients may abstain or diminish alcohol intake before visiting a clinic or physician. Thus, the observed elevations in blood pressure could be due to excessive central-nervous-system excitability and adrenergic discharge associated with the withdrawal period.

Baroreceptors in alcohol-induced hypertension

Alcohol diminishes the baro (presso) reflex by interacting with receptors in the brain stem, i.e. nucleus tractus solitarius and rostral ventrolateral medulla^[43]. Other investigators reported that baroreceptor reflex curves, which indicate the gain in baroreceptor reflex sensitivity, were shifted up and reduced slope in ethanol fed rats when challenged with vasoconstrictors (phenylephrine and angiotensin II) compared with controls^[45]. These findings and others^[42,46,47] suggest the impairment of baroreceptor control and sympathetic system. A greater decrease in heart rate in ethanol treated rats compared with control rats during β -adrenoreceptor blockade with propranolol indicates that the ethanol treated rats had an increased sympathetic activity. An increase in sympathetic activity is consistent with impairment of the baroreceptors that, when activated, inhibit the sympathetic nervous system^[45,47]. However this mechanism is implicated more likely in acute alcohol-induced hypertension.

Sympathetic nervous system in alcohol-induced hypertension

Several studies reported increased sympathetic nervous system activation and discharge of sympathetic amines after alcohol consumption^[43,48,49]. Alcohol may cause hypertension by affecting the autonomic nervous system^[50]. However, alterations in the sympatho-adrenal function that occur during ageing may cause older people to have a different reaction to factors triggering their autonomic system than do younger individuals^[51]. The increased sympathetic outflow is expected not only to induce adrenoreceptor-mediated reactions (vasoconstriction, heart rate increase) but to stimulate oxidation reactions^[43]. Direct recordings of sympathetic-nerve activity suggest that short-term alcohol ingestion in humans and both short and long-term administration of ethanol in rats stimulates sympathetic-nerve discharge^[47,49,50]. Moreover, in rats the alcohol-induced increases in blood pressure and sympathetic activity is centrally mediated^[47]. It is possible that alcohol may stimulate adrenals to release adrenaline, resulting in increased heart rate, cardiac output and systolic blood pressure^[52]. Randin *et al.*^[53] have also reported that alcohol induces hypertension in rats by sympathetic activation that appears to be centrally mediated. This mechanism is also likely being implicated in alcohol-induced hypertension.

Renin-angiotensin-aldosterone system in alcohol-induced hypertension

The serum levels of vasoactive substances such as renin-aldosterone have been reported to be affected by alcohol ingestion *in vivo* or ethanol *in vitro*^[54-56]. Antihypertensive drugs are shown to offer protection against alcohol

induced responses in cultured human endothelial cells suggesting the possible involvement of renin-angiotensin system (RAS)^[56]. It has been reported that a significant increase in plasma renin activity in patients consuming heavy alcohol compared to mild or moderate alcohol consumption^[55,57,58]. However other reports showed no significant increase in plasma renin activity after alcohol consumption^[48,59]. Other studies reported an expansion of the extracellular fluid after alcohol consumption which has been shown to elevate the systolic blood pressure in rats^[60,61]. Chan *et al.*^[60] have proposed that expansion of the extracellular fluid is the result of elevated plasma vasopressin levels and plasma renin activity, indicating increased sympathetic stimulation. Recent studies have shown a significant increase in blood and aortic angiotensin II levels after alcohol ingestion in rats^[62,63]. Okuno *et al.*^[64] have reported prolonged elevation of serum angiotensin converting enzyme (ACE) activity in alcoholics suggests that angiotensin II levels are elevated due to activation of ACE activity. Alcohol ingestion in dogs caused sustained RAS activation with progressive increases in plasma levels of Angiotensin II, renin activity, left ventricular ACE enzyme activity, and left ventricular myocyte Ang II AT1 receptor expression^[65]. This mechanism is more likely implicated in alcohol-induced hypertension.

Cortisol in alcohol-induced hypertension

Certain studies have implicated the role of cortisol in alcohol-induced rise in blood pressure^[66-68]. Potter *et al.*^[66] have reported a significant rise in plasma cortisol levels following alcohol consumption and a drop in plasma cortisol levels when alcohol intake was discontinued. Increased cortisol levels in regular alcohol drinkers may be due to direct stimulation of adrenocorticotropin hormone or potentiation of corticotropin releasing hormones by arginine vasopressin^[67]. The effect of blood pressure may be due to the mineralocorticoid activity of cortisol or catecholamine hypersensitivity^[68]. Alcohol stimulates the secretion of corticotrophin releasing hormone in rats^[69,70] leading to stimulation of cortisol secretion^[71], sympathetic stimulation and hypertension in rats. However this mechanism is implicated more likely in acute alcohol-induced hypertension.

Increased intracellular calcium and vascular reactivity in alcohol-induced hypertension

Rats treated with ethanol showed constriction of blood vessels^[72] due to greater shifts in the binding of the calcium ion (Ca^{2+}) in arterial and arteriolar smooth muscle cells causes increased sensitivity to endogenous vasoconstrictors. This finding is consistent with other reports showing the shifts of the extracellular Ca^{2+} to intracellular space increase the vascular sensitivity to vasoconstrictor norepinephrine^[50,61]. It is proposed that alcohol increases intracellular Ca^{2+} by (1) direct upregulation of voltage-gated Ca^{2+} channels; (2) inhibition of Ca^{2+} -adenosine triphosphatase (Ca^{2+} -ATPase) that extrudes Ca^{2+} from the cells; and (3) magnesium ion (Mg^{2+}) depletion that inhibits the sodium ion (Na^{+})-potassium

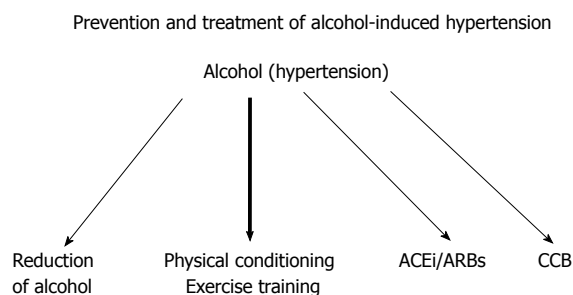


Figure 2 Prevention and treatment of alcohol-induced hypertension. ACEi/ARBs: Angiotensin converting enzyme inhibitor/Angiotensin receptor blockers; CCB: Calcium channel blockers.

ion (K^+) pump (Na^+/K^+ -ATPase), causing a build up of intracellular Na^+ . This reaction in turn inhibits the Na^+/Ca^{2+} exchanger, thereby increasing the intracellular calcium ion^[50,61,72,73]. Chronic alcohol ingestion has been reported to induce a deficiency of blood and intracellular magnesium, which influences cellular Ca^{2+} homeostasis through attenuation of plasmalemmal ATPase activity^[74]. Vasdev *et al.*^[75] have shown that increases in cytosolic free calcium and calcium uptake are associated with ethanol-induced hypertension in rats. Intra-arterial infusion of ethanol has been shown to reduce hand and forearm blood flow in humans^[76]. This effect could be the result of a direct vasoconstriction or of a loss of endothelium dependent vasorelaxation^[77]. However earlier studies in rats demonstrated no significant response of alpha-adrenergic receptor-mediated constriction of aorta after chronic ethanol ingestion in rats^[45,78-80]. On the other hand, the endothelium-dependent relaxation elicited by acetylcholine was diminished in chronic alcohol-induced hypertension^[77]. Our earlier study also demonstrated the role of endothelium-independent responses in the aorta of chronic alcohol treated hypertensive rats^[79,80]. Inconsistencies among several reports render this mechanism of alcohol-induced hypertension less implicated.

Endothelium and oxidative stress in alcohol-induced hypertension

Imbalance of specific endogenous vasoconstrictor such as angiotensin II, endothelin-1 and nor-epinephrine and vasodilator nitric oxide (NO) may also play an important role in alcohol-induced hypertension. Alcohol stimulates the release of endothelin 1 and 2 from vascular endothelium in a dose dependent manner^[81]. Alcohol also increases the angiotensin II levels in the blood and vessels^[62,63]. Endothelin 1 and 2 as well as angiotensin II are known to be potent vasoconstrictors of the blood vessels^[63,81]. Angiotensin II stimulates superoxide production via AT_1 receptor, by activating NADPH oxidase in the vascular wall^[82,83]. Superoxide productions through NADPH oxidase activation ($p22^{phox}$ expression) has been demonstrated in rats made hypertensive with angiotensin II infusion^[84]. Chronic ethanol ingestion induces hypertension which is correlated with elevated tissue angiotensin II levels, and activation of NADPH oxidase activity causing endothelial injury in rats^[62,79,80]. It is pos-

sible that alcohol ingestion raises the blood pressure by decreasing the vasodilators such as NO in the vascular endothelium either due to inhibition of endothelial nitric oxide synthase (eNOS) or inflammatory/oxidative injury to the endothelium. Earlier studies have also shown that chronic ethanol consumption either interferes with NO production or release of NO from endothelial cells^[80,85-87]. The diminished NO bioavailability may either be related to reaction with superoxide anion to form peroxynitrite radicals^[88] or oxidative inactivation/uncoupling of eNOS by ethanol-induced free radicals^[80,89,90]. The production of NO in the endothelium is critically dependent on the function of eNOS which is regulated by vascular endothelial growth factor^[91,92]. Alcohol inhibits the enzyme that converts arginine into NO^[93] as well as eNOS protein expression^[80]. In the endothelium, depletion of NO production or NO reaction with superoxide anion to form toxic peroxynitrite radical which causes endothelial injury, impairment and hypertension in alcohol treated rats^[20-22,62,80,94]. Recent studies have shown that chronic ethanol ingestion induces hypertension which was related to increased aortic inflammation, elevated angiotensin II levels, induction of NADPH oxidase causing endothelial injury, depletion of antioxidants, down-regulation of endothelial NO generating system and impaired vascular relaxation in rats^[6,19-22,62,80]. This mechanism is most likely implicated in chronic alcohol-induced hypertension.

PREVENTION AND TREATMENT OF ALCOHOL-INDUCED HYPERTENSION

There are few strategies for the control, prevention and treatment of alcohol-induced hypertension as shown in Figure 2.

Studies have shown that a reduction in alcohol intake is effective in lowering the blood pressure both in hypertensives and normotensives and may help to prevent the development of hypertension^[12,41,95,96]. Heavy drinkers who cut back to moderate drinking can lower their systolic blood pressure by 2 to 4 mm of mercury (mm Hg) and their diastolic blood pressure by 1 to 2 mmHg. Heavy drinkers who want to lower blood pressure should slowly reduce how much they drink over one to two weeks.

Another non-pharmacological prevention and treatment of alcohol-induced hypertension is physical conditioning or exercise training. There is a physiological basis for effect of physical conditioning on chronic alcohol-induced hypertension in a rat model. Exercise increases the utilization of oxygen in the body and up-regulate the antioxidant defense system in the cardiovascular system^[97-100]. Exercise training also generates NO in the cardiovascular system by induction of nitric oxide synthase^[19,79,90,101]. Recent studies have shown the beneficial role of physical training in the control of blood pressure in humans^[97,98,102,103] and experimental animals^[79,90,104,105]. Physical inactivity and overweight trigger hypertension^[106,107] whereas; regular physical activity has been shown to decrease the BP and body weight^[102,103]. Stud-

ies have shown that physical conditioning is beneficial in lowering the BP through suppression of weight gain in chronic ethanol treated hypertensive rats^[19,79]. Physical conditioning attenuates the chronic ethanol-induced hypertension by augmenting the NO bioavailability and reducing the oxidative stress response in rats^[19,79,108].

PHARMACOLOGICAL TREATMENT OF ALCOHOL-INDUCED HYPERTENSION

There are no definite clinical data available on the efficacy of specific drugs in the treatment of alcohol-induced hypertension. Randin *et al.*^[53] have reported that dexamethasone (2 mg per day) in human suppresses the acute alcohol-induced hypertension. It is suggested that ACE inhibitors/angiotensin II receptor type 1 (AT₁) blockers, because of their ability to increase the cardiac output in patients with alcohol-induced cardiomyopathy will be useful in the treatment of alcohol-induced hypertension. Cheng *et al.*^[65] have shown that angiotensin II type 1 receptor blockade prevents alcoholic cardiomyopathy in dogs. The calcium channel blockers, because of the probability of the involvement of calcium in the development of alcohol-induced hypertension, may also likely be the drug of choice for the treatment of alcohol-induced hypertension.

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WJC 6th Anniversary Special Issues (1): Hypertension

Pediatric hypertension: An update on a burning problem

Pier Paolo Bassareo, Giuseppe Mercurio

Pier Paolo Bassareo, Giuseppe Mercurio, Department of Medical Sciences "Mario Aresu", University of Cagliari, Cagliari 09042, Italy

Author contributions: Bassareo PP wrote the manuscript; Mercurio G critically revised the manuscript and provided final approval.

Correspondence to: Pier Paolo Bassareo, MD, PhD, Department of Medical Sciences "Mario Aresu", University of Cagliari, Policlinico Universitario, S.S. 554 bivio di Sestu, Monserrato, Cagliari 09042, Italy. piercard@inwind.it

Telephone: +39-07-06754953 Fax: +39-07-06754953

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Abstract

A large number of adults worldwide suffer from essential hypertension, and because blood pressures (BPs) tend to remain within the same percentiles throughout life, it has been postulated that hypertensive pressures can be tracked from childhood to adulthood. Thus, children with higher BPs are more likely to become hypertensive adults. These "pre-hypertensive" subjects can be identified by measuring arterial BP at a young age, and compared with age, gender and height-specific references. The majority of studies report that 1 to 5% of children and adolescents are hypertensive, defined as a BP > 95th percentile, with higher prevalence rates reported for some isolated geographic areas. However, the actual prevalence of hypertension in children and adolescents remains to be fully elucidated. In addition to these young "pre-hypertensive" subjects, there are also children and adolescents with a normal-high BP (90th-95th percentile). Early intervention may help prevent the development of essential hypertension as they age. An initial attempt should be made to lower their BP by non-pharmacologic measures, such as weight reduction, aerobic physical exercise, and lowered sodium intake. A pharmacological treatment is usually needed should these measures fail to lower BP. The majority of antihypertensive drugs are not formulated for pediatric

patients, and have thus not been investigated in great detail. The purpose of this review is to provide an update concerning juvenile hypertension, and highlight recent developments in epidemiology, diagnostic methods, and relevant therapies.

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Key words: Children; Hypertension; Blood pressure; Epidemiology; Diagnosis; Therapy

Core tip: It is generally presumed by cardiologists that arterial hypertension is a disease that typically develops only in adult life. However, a number of studies testify that this pathologic process can begin early in childhood, as evidenced by occasional increases in blood pressure (BP) or abnormal BP responses to physical or psychological stress. This review provides a detailed analysis concerning the epidemiology, diagnostic methods, and therapies for pediatric hypertension.

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INTRODUCTION

Although secondary arterial hypertension (HTN) was thought to be more frequent than essential arterial HTN in children, recent reports indicate that essential HTN is the most frequently manifested form of the disease during both childhood and adolescence^[1]. Pediatric HTN is now commonly known worldwide to be an early risk factor for cardiovascular morbidity and mortality. The essential HTN subsequently detected in adults may have already been manifested at an early age, observed as occasional raises in blood pressure (BP) or abnormal BP response to physical or psychological stress. Similar to

other types of chronic illness, the hypertensive process likely develops several decades prior to the onset of clinical signs and symptoms^[2,3]. As BP levels are typically retained throughout life, children with higher BPs are more likely to become hypertensive adults^[4].

Extensive normative data on BP in children have long been available both in the United States and Europe. Pediatric BP nomograms were developed by the Task Force on BP Control in Children, commissioned by the National Heart, Lung, and Blood Institute of the National Institutes of Health, by using results obtained from 83000 children and adolescents of both genders. The percentile curves were first published in 1987 and described age-specific distributions of systolic and diastolic BPs for an age range between 5 and 17 years, with corrections for height and weight^[5]. The third report from the Task Force, published in 1996, provided additional details regarding the diagnosis and treatment of HTN in infants and children^[6]. In 2004, the fourth report added further information and adapted the data to growth charts previously developed from the Centers for Disease Control and Prevention^[7]. In an update to the HTN guidelines published by the European Society of Cardiology in 2009, a new chapter was devoted to HTN in children, with an approach similar to the American version^[8].

In accordance with the recommendations of the Task Force, BP is considered normal when the systolic and/or diastolic values are less than the 90th percentile for the child's age, sex, and height. BP is considered high for systolic and/or diastolic values > 95th percentile. For BPs between the 90th and 95th percentile, a new category (pre-HTN) has been introduced, defined as a BP \geq 120/80 mmHg. In cases where systolic and diastolic pressures are discrepant with respect to classification, the child's condition should be categorized using the higher value^[7].

BP usually depends on the balance between cardiac output and vascular resistance. BP increases following a rise in either of these variables without a compensatory decrease from the other^[9]. Factors affecting cardiac output include the following: baroreceptors, extracellular volume, effective circulating volumes of atrial natriuretic hormones, mineralocorticoids, and angiotensin, as well as contributions from the sympathetic nervous system^[10]. Factors influencing vascular resistance include pressors, such as angiotensin II, calcium (intracellular), catecholamines, vasopressin and the sympathetic nervous system, as well as depressors, such as atrial natriuretic hormones, endothelial relaxing factors, kinins, prostaglandin E₂ and prostaglandin I₂^[10].

Changes in electrolyte blood concentrations (particularly changes in sodium, calcium, and potassium levels), may also affect vascular resistance. Under normal conditions, extracellular volume is maintained by the excretion of sodium in amounts equal to those ingested. Retention of sodium results in an increase in extracellular volume, and an elevation of BP. Sodium balance is restored by renal changes in both the glomerular filtration rate and the tubular reabsorption of sodium, resulting in natriuretic excretion of excess sodium. Elevated calcium concentra-

tions can increase vascular smooth cell contractility, and stimulate the release of renin, synthesis of epinephrine, and enhance sympathetic nervous system activity and increasing BP. Reduced potassium intake can also increase BP by stimulating the production and release of renin and reducing natriuresis. The renin-angiotensin system and the hypothalamus-hypophysis-adrenal gland axis are suspected to be involved in the elevation of BP as well^[11,12]. This complexity demonstrates the difficulty in identifying the mechanism that accounts for HTN, and explains why treatment is often designed to affect regulatory factors rather than the cause of the disease. For example, BP can be elevated as a result of increased sodium renal reabsorption, insulin resistance, leptin resistance, vascular resistance, and sympathetic tone caused by hyperinsulinemia.

EPIDEMIOLOGY

The prevalence of HTN in the pediatric population was examined as early as 1963^[13], though the precise rates are not known. The majority of studies report rates ranging from 1% to 5%, although prevalence as high as 10% has been reported for some isolated geographic areas^[14-19]. However, regression to the mean from repeated measurements included from more recent studies has placed the prevalence of HTN at less than 5%^[19]. The discrepancy in reported values is likely due, in part, to the arbitrary definition of HTN and the BP measurement method^[20]. The frequent use of non-specific population BP nomograms may exaggerate the prevalence of HTN in children and adolescents in some specific geographic areas. In fact, genetic and environmental differences can influence HTN incidence between regions. Although reference standards established in the US have been adopted worldwide, many local percentile curves are still being used, especially in northern Europe^[14,16,20,21], and clinicians from every ethnic group or geographic area in the world should produce their own national BP nomograms relating to age, gender, and height.

A review by Chiolerio *et al*^[13] examined the HTN prevalence rates reported in large-scale school-based studies (> 2000 children) from all over the world published between 1980 and 2006. Most studies determined HTN from a single BP measurement, with a prevalence of isolated systolic HTN at 7.2%-19.4%. However, in the only study where three different BP measurements were used, the overall prevalence of HTN was 4.5%. While some authorities recommend only one recording, others advocate taking the average of two or three pressures, which more accurately reflects the overall BP of the individual patient^[22]. According to the United States Task Force, elevated pressures must be confirmed on repeated visits before characterizing a child as having HTN, with at least three different measurements strongly recommended^[7,14]. The prevalence of pediatric HTN can also be influenced by the method of measurement, as oscillometric devices vary by manufacturer and require validation and calibration, and auscultation is subject to operator-dependent

biases such as rounding errors (digit preference), expectation bias, and operator skill^[23].

Recent reports on the prevalence of normal-high BP or pre-HTN (between 90th and 95th percentile) in younger individuals is concerning, as it is associated with an intermediate degree of organ damage^[24]. In three different recent surveys performed in the United States, the prevalence of normal-high BP ranged from 3.4% to 31.4% in large cohorts of children and adolescents, which was largely influenced by age and weight^[25-27]. In a study on high school students by McNiece *et al*^[28], the prevalence of combined pre-HTN and HTN was over 30% in obese boys and 23%-30% in obese girls, depending on ethnicity. A three-year longitudinal screening of BP in Italy found that pediatric pre-HTN and HTN are equally prevalent^[29,30].

DIAGNOSTIC TOOLS

Two non-invasive methods, auscultatory and oscillometric, are typically used to diagnose HTN in children and adults. When the auscultatory method is used, pediatric systolic BP is defined on the basis of the first Korotkoff sound, while the diastolic BP usually corresponds to the fifth Korotkoff sound^[31]. However, a meta-analysis from the Bogalusa Heart Study indicated that the fourth Korotkoff sound is a more reliable measure of diastolic BP and a better predictor of adult HTN than the fifth^[32]. Moreover, a comparison of these methods for BP measurement in the San Antonio Triethnic Children's BP Study indicated that systolic and diastolic pressure readings were 10 and 5 mmHg higher, respectively, with an oscillometric compared to an auscultatory device^[33]. Thus, caution must be used when diagnosing HTN with an automated device.

The current United States Task Force recommendation for choosing an appropriate size cuff for measuring BP is a bladder width equal to 40% of the upper arm circumference (UAC). However, most physicians use the older two-thirds or three-fourths upper arm length (UAL) recommendations to choose a cuff, and significant differences have been highlighted between the methods. Specifically, systolic BP measured using the 40% UAC criterion reflects a directly measured radial arterial pressure and significantly overestimates the diastolic pressure. Using available cuffs for indirect measurements by two-thirds and three-quarters UAL criteria significantly underestimates systolic as well as diastolic BPs when compared with radial intra-arterial BP^[34]. Therefore, recommendations for BP cuff selection should be reviewed. Moreover, labeling of BP cuffs for infant, pediatric, small adult, adult, and large adult patients is misleading, and such designations should be eliminated. Cuff sizes should be standardized, indicate bladder size, and be uniformly color-coded for convenience^[35].

Twenty-four-hour ambulatory BP monitoring (ABPM) can more precisely characterize changes in BP during daily activities, and is superior to clinical BP monitoring in predicting cardiovascular morbidity and mortality in adults^[36,37]. As children and adolescents tend to be more

emotional with consequent BP raises that can indicate HTN, ABPM may differentiate those with "white coat" HTN from those with chronic HTN. As a result, ABPM is gaining acceptance as a useful modality for the evaluation of BP levels in research and clinical settings^[38,39], and may help overcome some of the challenges clinicians face when attempting to categorize a young patient's BP levels^[40]. ABPM is recommended for the standard assessment of pediatric patients for confirming the diagnosis of HTN (*e.g.*, exclusion of "white coat" HTN), evaluating for the presence of masked HTN, assessing BP variability, determining dipping status in patients at high risk for larger organ damage (*e.g.*, those suffering from sleep apnea), assessing the severity and persistence of HTN, and evaluating BP levels in chronic pediatric diseases associated with HTN. In addition, ABPM can be used to evaluate the effectiveness of drug therapy, monitor for drug-resistant HTN, and determine whether symptoms are a result of drug-related hypotension.

PEDIATRIC BP MONITORING

For monitoring BP in children, a suitable ABPM device should be selected, such as devices with appropriate cuff sizes that have been validated according to the standards set by the Association for Advancement in Medical Innovation or the British Heart Society. Moreover, individuals with specific training in the application and interpretation of ABPM data in pediatric patients should obtain the readings using a standard approach. Monitors should be applied to the non-dominant arm unless contraindicated (*e.g.*, the presence of an arteriovenous fistula). After application, results should be compared with resting BP measured in the clinic using the same technique as used by the ambulatory device (auscultatory or oscillometric). Calibration between methods should be considered adequate when there is agreement within 5 mmHg between the average of three clinic and three ambulatory BP measurements. Cuff placement and proper device function should be verified for values falling outside this range. Wide disagreement between resting and ambulatory device measurements of diastolic BP may occur with the use of auscultatory ABPM devices that lack pediatric settings to adjust for the larger fourth and fifth Korotkoff sound differences often seen in younger children. If this occurs, an oscillometric device may be preferred, or interpretation may be restricted only to the values for systolic BP.

Patients or their guardians should be instructed to record their antihypertensive medication intake, and activity, sleep, and wake times in a diary. As a sufficient number of valid BP recordings are needed to provide interpretable data, devices should be programmed to record BP every 20 to 30 min during waking hours and every 30 to 60 min during sleep hours. ABPM recordings should be edited for outlying values and data should be visually inspected for gross inconsistencies, such as BPs and heart rates that fall considerably outside the ranges normal for the patient's age, such as a systolic BP 60 to 220 mmHg,

diastolic BP 35 to 120 mmHg, heart rate 40 to 180 beats per minute, or pulse pressure 40 to 120 mmHg. As a general rule, the above stated limits should be programmed into the ABPM software to minimize subjective editing of ABPM data. Standard calculations should be reported (mean ambulatory systolic and diastolic BP during the 24 h, daytime, and nighttime periods). Dipping (percent day-night difference) should be determined for systolic and diastolic pressures: $(\text{mean daytime BP} - \text{mean nighttime BP}) / \text{mean daytime BP} \times 100$. ABPM levels should be interpreted using appropriate pediatric normative data, such as gender- and height-specific data obtained from large pediatric populations using similar techniques. A diagnosis of HTN is indicated by significant abnormalities in ambulatory BP levels and loads occurring during the daytime, nighttime, or the entire 24-h period^[41,42].

THERAPY

An initial attempt should be made to lower BP by means of non-pharmacologic procedures, in spite of scientific evidence underlining the limited efficacy of this type of approach. There is a strong association between BP values and body weight, and weight loss in children is correlated with lowered BPs^[43-48]. Therefore, the primary objective should be to achieve and maintain a normal body weight. Regular exercise and a reduction in sedentary activities (such as watching TV or playing video-games) will result in enhanced weight loss and improved BP values^[49]. In addition, the intake of sugary drinks and calorie-rich foods should be limited and fresh fruits and vegetables encouraged, to ensure a satisfying and healthy diet. The help of a dietician specializing in the treatment of children and adolescents may be particularly useful in motivating self-control^[50-52].

Sodium intake should also be limited. Many studies have reported that a reduced-sodium diet decreases BP values in children by 1-3 mmHg^[53-58]. A randomized trial demonstrated that adolescent BPs were significantly reduced by limiting sodium intake in early childhood^[59]. Current recommendations for sodium intake are 1.2 g/d for children between the ages of 4 and 8 years, and 1.5 g/d for older children^[60], which are lower than the amount of sodium present in a typical daily diet. Thus, a reduction in salt intake together with a reduced-calorie diet may enhance the effects achieved by weight loss alone^[7]. Other lifestyle changes, such as improving the quality of sleep or quitting smoking, can also help to lower BP^[61,62].

Pharmacologic treatment is indicated for HTN that persists despite these lifestyle changes, as well as for secondary HTN, HTN associated with organ dysfunction, and HTN in diabetic patients (type 1 and type 2), according to United States guidelines^[7]. In addition, children or adolescents with dyslipidemia, although not included in the therapeutic indications, may also benefit from administration of a low-dose antihypertensive therapy^[63,64]. The main therapeutic aim is to reduce BP to below the 95th percentile, or below the 90th percentile if other cardiovascular risk factors are present. The number of antihyper-

tensive drugs specifically indicated for use in children has risen considerably in recent years, including beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers, calcium channel blockers, and diuretics. Trials using these drugs in children have been directed almost exclusively at assessing their efficacy in lowering BP, and show these drugs to be safe and well-tolerated with satisfactory short-term BP reduction^[7]. Several classes of drugs are particularly indicated for use in hypertensive subjects with specific concomitant diseases. As an example, ACE inhibitors and angiotensin-receptor blockers are recommended for hypertensive diabetics or those with microalbuminuria, as well as in patients with chronic renal failure and proteinuria, whereas beta-blockers and calcium channel blockers are indicated for use in patients affected by migraine and headache. The lowest dose of antihypertensive drug should be used and gradually increased until the desired BP values are achieved. If peak doses are reached without any appreciable benefit, or the young patient manifests adverse effects, it may be advisable to implement a combined therapy with a second drug that enhances the efficacy of the first^[7]. Particular care should be taken in monitoring children for organ dysfunction and potential adverse effects, as well as in assessing the efficacy of treatment. For example, children undergoing treatment with ACE inhibitors and/or diuretics should be carefully monitored for electrolytic balance. Combination pharmacotherapy in children has not been well studied, and is not recommended as an initial treatment. Multi-drug combinations, such as bisoprolol and hydrochlorothiazide, should only be prescribed in particularly unresponsive and severe cases^[65]. A "step-down" therapy may be implemented in patients achieving satisfactory BP values over a lengthy period, such as a gradual tapering of treatment in overweight/obese children who have lost a significant amount of weight. In some cases, treatment may be withdrawn, though patients should undergo long-term follow-up to monitor for relapse^[7,66].

Pharmacologic treatment is inherently more difficult in children than it is in adults. Unlike children, adults are able to learn to live with their condition, maintain treatment compliance, and are mindful of the consequences of untreated HTN^[67]. The majority of drugs available for the treatment of HTN do not have pediatric formulations, and often assume a distasteful flavor when divided or pulverized, though thiazide diuretics (hydrochlorothiazide and chlorthalidone), calcium channel blockers (lercanidipine), and angiotensin receptor antagonists (candesartan) do not have any flavor and can therefore easily be administered to small children^[65,68]. Drugs that are prescribed for hypertensive children should have minimal side and prolonged therapeutic effects, though slow-release formulations should be avoided as they are poorly absorbed by children and lose their prolonged effect once the tablets are split. Adverse side effects are most frequent with diuretics, followed by beta-blockers, calcium antagonists, ACE-inhibitors and fast angiotensin receptor blockers^[65,68]. Moreover, similar to other classes

of compounds, antihypertensive drugs have different pharmacokinetics in children, particularly in very young children; therefore, drug dosage should be adjusted^[65,68].

CONCLUSION

Cardiovascular diseases such as HTN develop slowly and their pathogenesis often begins in childhood. A routine use of specific and carefully constructed BP tables will allow pediatric clinicians and cardiologists to identify pathophysiologic conditions in their patients that may only clinically manifest after several decades. The diagnosis and treatment of pediatric high BP and HTN should therefore be considered as a preventative measure, rather than simply the tracking of an early predisposition to a fatal destiny in adulthood.

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WJC 6th Anniversary Special Issues (1): Hypertension

Potential pathophysiological role for the vitamin D deficiency in essential hypertension

Federico Carbone, François Mach, Nicolas Vuilleumier, Fabrizio Montecucco

Federico Carbone, Fabrizio Montecucco, Department of Internal Medicine, University of Genoa School of Medicine, IRCCS Azienda Ospedaliera Universitaria San Martino-IST Istituto Nazionale per la Ricerca sul Cancro, 16132 Genoa, Italy

Federico Carbone, François Mach, Cardiology Division, Foundation for Medical Researches, Department of Internal Medicine, University of Geneva, 1211 Geneva, Switzerland

Nicolas Vuilleumier, Fabrizio Montecucco, Division of Laboratory Medicine, Department of Genetics and Laboratory Medicine, Geneva University Hospitals, 1205 Geneva, Switzerland

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Correspondence to: Dr. Fabrizio Montecucco, MD, PhD, Division of Laboratory Medicine, Department of Genetics and Laboratory Medicine, Geneva University Hospitals, 4 rue Gabrielle-Perret-Gentil, 1205 Geneva, Switzerland. fabrizio.montecucco@unige.ch

Telephone: +41-22-3827238 Fax: +41-22-3827245

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"local vitamin D system") and the link of vitamin D with renin-angiotensin-aldosterone system and the fibroblast growth factor 23/klotho pathways highlighted its active cardiovascular activity. Focusing on hypertension, this review summarizes the more recent experimental evidence involving the vitamin D system and deficiency in the cardiovascular pathophysiology. In particular, we updated the vascular synthesis/catabolism of vitamin D and its complex interactions between the various endocrine networks involved in the regulation of blood pressure in humans. On the other hand, the conflicting results emerged from the comparison between observational and interventional studies emphasize the fragmentary nature of our knowledge in the field of vitamin D and hypertension, strongly suggesting the need of further researches in this field.

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Key words: Vitamin D; Hypertension; Cardiovascular disease; Renin; Angiotensin

Core tip: This review provides a comprehensive and critical analysis of the most recent studies investigating the relationship between vitamin D and essential hypertension. From the both observational and interventional studies, conflicting results have been shown. This review article provides some hypothesis to explain these discrepancies. In addition to the potential bias related to the study design, some pathophysiological explanation was suggested, especially involving the potential role of local vitamin D system as well as the fibroblast growth factor 23/klotho axis. This review aims at suggesting a careful reflection so that future studies might be designed for minimize bias and encompass the complex biology of vitamin D system.

Abstract

Vitamin D deficiency has been indicated as a pandemic emerging public health problem. In addition to the well-known role on calcium-phosphorus homeostasis in the bone, vitamin D-mediated processes have been recently investigated on other diseases, such as infections, cancer and cardiovascular diseases. Recently, both the discovery of paracrine actions of vitamin D (recognized as

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INTRODUCTION

Vitamin D deficiency has recently emerged as a public health problem, affecting almost 50% of the population worldwide^[1]. In addition to the reduced exposition to sunlight^[2], also genetic and environmental factors have been suggested as a cause of this pandemic, such as pollution, diet, sedentary life style and stress^[3]. Moreover, vitamin D is no longer considered as only a pivotal mediator of calcium metabolism and skeletal health, but it also regulates several cell functions, including differentiation and metabolism. This aspect may explain the reason why hypovitaminosis D has been proved to be an independent risk factor for overall mortality in various cohort analyses^[4], whereas vitamin D supplementation significantly reduced mortality^[5]. Moreover, similar data were collected from different clusters of inflammatory and chronic diseases, such as infections^[6], autoimmunity^[7], neurodegenerative pathologies^[8], as well for cancer^[9]. However, a special interest was conferred to the potential relationship between vitamin D and cardiovascular (CV) disorders. Although in human cohorts low vitamin D levels were associated with impaired CV outcomes^[10], a causal relationship remains unknown, and the general enthusiasm about the benefits of vitamin D supplementation have been recently replaced by words of caution.

On the other hand, novel topics that might address many question in the field of vitamin D, such as fibroblast growth factor (FGF) 23-klotho axis, non-genomic effects of vitamin D and the paracrine effects of vitamin D (also called “local vitamin D system”) have been identified. In the following paragraphs, we will focus on the mechanisms triggered by vitamin D in arterial hypertension, starting from the complex interplay with the renin-angiotensin-aldosterone system (RAAS) in both basic research and clinical trials.

VITAMIN D SYSTEM AND BLOOD PRESSURE

Vitamin D

In humans, more than 80% of vitamin D requirements is produced through the ultraviolet-B (UVB)-induced conversion of 7-dehydrocholesterol to vitamin D in the skin, whereas only 10%-20% is absorbed with the diet^[1]. The photosynthesis of vitamin D evolved over 750 million years ago, first in the phytoplankton and then in early plants and animals^[11]. From an evolutionary stand point it is interesting to note that the first living beings synthesizing vitamin D were missing calcific skeleton. This suggests that a new recognized non-metabolic role (called “non-classical effects”) of vitamin D might actually be the oldest. Regardless of the source, vitamin D requires liver hydroxylation [through 25-hydroxylase (CYP2R1 or

CYP27A1)] to form 25-hydroxyvitamin D [25(OH) vitamin D or calcidiol], inactive form but used as reference for vitamin D status, because abundant, stable and easier to quantify^[1]. In the kidney 25(OH) vitamin D is then hydroxylated to 1,25-dihydroxyvitamin D [1,25(OH)₂ vitamin D or calcitriol] the active form of vitamin D [through 25-hydroxyvitamin D-1 α -hydroxylase (CYP27B1)]. This latter step is a pivotal effector of calcium homeostasis and thus highly controlled by the up-regulation of parathyroid hormone (PTH) and the suppression of FGF23/klotho axis^[12]. Although the exact contribution of extra-renal hydroxylation in determining the circulating levels of 1,25(OH)₂ vitamin D is still unknown, it has been recognized also an extra-renal activity of CYP27B1. Finally, the recent identification of a role of vitamin D binding proteins on vitamin D catabolism has further increased complexity of the system^[13].

Vitamin D receptor

Vitamin D receptor (VDR) is member of nuclear hormone receptors superfamily. Following binding with 1,25(OH)₂ vitamin D, VDR recruits one of the retinoid X receptors (RXR α , β or γ) forming homo- or heterodimers to promote a specific, high-affinity DNA-binding interaction. This transcriptional complex binds to repeated sequences of 6 hexamers [vitamin D response elements (VDRE)] in the promoter region of target gene^[1]. VDR is believed to directly or indirectly regulate 3% to 5% of human genome and the different genomic activation of vitamin D in the different cell types involves allosteric influences, VDRE location and epigenetic modification (of both DNA and histones)^[14]. In addition, VDR recognizes extra-nuclear ligands including endogenous steroids and other lipophilic compounds^[15,16]. Finally, VDR may be expressed also on the cell surface membrane and within mitochondria thus might modulate non-genomic signalling pathways, such as 1,25(OH)₂ vitamin D-mediated rapid-response^[17]. Vitamin D are deeply involved in several patterns of CV pathophysiology, including vascular inflammation^[18] and endothelial dysfunction^[19] as observed in patients with chronic kidney disease (CKD)^[20] and type 2 diabetes^[21] as well as in asymptomatic subjects^[22]. For instance, in vitro VDR activation induces nitric oxide production in endothelial cells^[23] and improves the angiogenic properties of endothelial progenitor cells^[24], while regulates proliferation^[25], migration^[26], mineralization^[27] and thrombogenic protein expression^[28] in vascular smooth muscle cells (VSMCs). The recent recognition of specific VDR polymorphisms and genetic susceptibility in pathophysiology of hypertension has further supported these insights^[29].

Vitamin D hydroxylases

The gene encoding for CYP27B1 is widespread expressed in various tissue of endodermal, ectodermal and mesenchymal origin. Since even VDR is highly represented in tissues, an autocrine/paracrine vitamin D system has been strongly suggested. In contrast to endocrine vitamin D system, local regulation of 1,25(OH)₂ vitamin D levels

is independent of PTH expression, but rather relies on environmental factors^[30]. CYP27B1 expression in endothelial cell is regulated by pro-inflammatory cytokines^[31], in VSMCs is under estrogenic control^[32] whereas many signals regulate the expression in monocyte/macrophage, including toll-like receptor^[33], interferon- γ ^[34], FGF23^[35] and uremia^[36]. Accordingly, CYP27B1^{-/-} mice develop an hypertensive phenotype, also characterized by increased circulating level of renin, angiotensin (Ang) II and aldosterone, then suppressed by administration of 1,25(OH)₂ vitamin D independently of serum levels of calcium or phosphorus^[37].

Vitamin D and FGF23/Klotho pathways

Recently, the discovery of FGF23 has extended the complexity of the endocrine network involving the vitamin D system. As vitamin D counter-regulatory hormone, FGF23 suppresses renal synthesis of 1,25(OH)₂ vitamin D by inhibiting CYP27B1 and up-regulating CYP24A1. These effects are independent of VDR but require co-factor klotho, essential for FGF23 signal transduction^[38]. Overall, 1,25(OH)₂ vitamin D and FGF23 are involved in a classical hormonal loop also including PTH. High levels of 1,25(OH)₂ vitamin D raise the serum concentrations of both calcium and phosphate. Concomitantly, the feedback by PTH reduces only calcium levels by enhancing its urinary excretion. Increased levels of FGF suppress the expression of sodium-phosphate cotransporter NaPi-2a on renal proximal tubules, thus resulting in increased phosphaturia^[39]. Therefore, phosphorus homeostasis might be maintained by 1,25(OH)₂ vitamin D *via* a direct regulation on FGF23 levels.

Thus, the discovery of FGF23 might explain some paradoxical concerns on vitamin D, especially among the ambiguous results of interventional studies. A strong correlation between an increased risk of mortality and high circulating levels of both FGF23 and phosphate has been also reported^[40,41], suggesting that there is a threshold in vitamin D supplementation beyond which 1,25(OH)₂ vitamin D may have detrimental effects.

For instance, the age-associated suppression of Klotho expression^[42] may promote a vitamin D toxicosis during therapeutic supplementation characterized by over-hyperphosphatemia and thus increased cardiovascular risk^[43]. Although it is likely a failure of the normal feedback mechanism regulating vitamin D and FGF23, the molecular bases of these clinical features have not been identified yet. Furthermore, Camalier *et al.*^[44] recently provided evidence of both rapid and late effects induced by FGF23 on mesenchymal stromal cells, involving cell proliferation and extracellular matrix (ECM) regulation. In addition, Jimbo *et al.*^[45] showed that FGF23 promoted osteoblastic differentiation of aortic VSMCs from uremic rats by inducing ERK1/2 phosphorylation pathway. However, it should be noted that these features were shown only in primary rat VSMCs and other studies failed to recognize the relevance of FGF23-Klotho signalling in mouse arteries^[46,47].

Ultimately, although further studies in humans are

warranted, we agree with Glade M.J., who suggested that there may be an age at which vitamin D deficiency may become life-sustaining, not life-threatening^[48].

PATHOPHYSIOLOGICAL PATHWAYS OF VITAMIN D IN HYPERTENSION

Although the effects of vitamin D on blood pressure have been known for several decades, some physiological aspects on the modulation of vascular cells and the vascular tone still remain to be clarified.

RAAS

RAAS plays a pivotal role in maintaining sodium and blood volume homeostasis even by modulating the renal function and blood pressure. RAAS up-regulation was shown to promote the development of hypertension and increased CV risk^[49,50].

Salt- and volume-independent RAAS up-regulation (documented by an increase in renin and Ang II levels) was associated with hypertension and cardiac hypertrophy in VDR^{-/-} mice^[51]. Similarly, in wild-type mice, 1,25(OH)₂ vitamin D inhibition (through dietary intake of strontium) increased renin expression, while 1,25(OH)₂ vitamin D supplementation down-regulated RAAS in a VDR-dependent manner^[51].

Also the evidence of a preserved CV function in VDR^{-/-} mice undergoing RAAS inhibition (using Angiotensin converting enzyme inhibitors or Angiotensin receptor I blockers) confirmed a direct connection between RAAS and vitamin D system^[52]. Interestingly, similar results were also reported in CYP27B1^{-/-} mice^[37]. Among the several cross-sectional and prospective studies investigating the association of vitamin D deficiency and hypertension only Forman *et al.*^[53] provided a mechanistic role of vitamin D system in the RAAS regulation. Lower 25(OH) vitamin D levels correlated with both higher Ang II at baseline ($P = 0.03$), and blunted renal plasma flow response to Ang II infusion in a cohort of 184 normotensive subjects treated with high-salt diet. These findings were confirmed in subsequent studies^[54,55].

From a molecular point of view, the research group directed by Li *et al.*^[52] discovered a direct effect of 1,25(OH)₂ vitamin D on renin gene transcription. They identified that vitamin D is capable of suppressing renin gene transcription by a cAMP response element, identified on the promoter region of *Ren-1c* gene^[56]. In addition, the same authors confirmed a central role of active vitamin D by excluding the control of PTH or serum calcium levels on renin expression^[57]. On the other hand, Ferder and co-workers have recently proposed a new hypothesis about the dependency instead of complementarity vitamin D system and RAAS. Overturning the classical view, the authors suggested the RAAS-induced inflammatory response as regulator of vitamin D status thus representing the “*primus movens*” of current vitamin D deficiency pandemic^[58]. Anyway, although suggestive, this hypothesis of a reciprocal counter-regulatory

effect between vitamin D and RAAS is currently highly speculative. Research models identifying effectors shared by RAAS and vitamin D are still missing^[59]. Angiotensin II is a main mediator responsible for adverse vascular remodelling in hypertension^[60]. By promoting endothelial dysfunction and vascular permeability, RAAS induces recruitment and activation of inflammatory cells within the vessel wall. This inflammatory behaviour stimulates hyperplasia and hypertrophy of VSMCs, but also their release of pro-inflammatory molecules (VCAM-1, monocyte chemoattractant protein-1, interleukin 6 and 8)^[61]. Furthermore, angiotensin II was shown to mediate the shift of VSMCs toward a fibroblast phenotype that alters the ECM composition by suppressing the activity of matrix metalloproteinases and enhancing the production of their inhibitors^[62]. Among the intracellular signalling pathways involved in angiotensin II signalling a key role is played by oxidants and their downstream signalling cascades including mitogen-activated protein kinase, protein kinase C, phospholipase A2 and the transcription factors NFκB and activator protein-1^[63].

PTH

PTH is a crucial regulator of calcium and phosphate homeostasis, achieved in different ways, such as osteoclast/osteoblast activation, enhancement of intestinal and renal calcium absorption and up-regulation of CYP27B1 expression in the kidney. Although not generally accepted^[64], higher PTH concentrations were associated with an increase in several CV risk factors^[65], including hypertension^[66-76]. Moreover, several cohorts of sporadic primitive hyperparathyroidism were found associated with arterial stiffness^[77-84]. The mechanism linking PTH and blood pressure is still unclear and several pathways might be triggered. PTH up-regulates RAAS activity promoting renin release^[85,86], but it also directly promotes aldosterone release from adrenal glands^[87]. Also the increase of serum calcium PTH may indirectly modulate renin release^[88] and aldosterone synthesis^[87] in addition to activate VSMC^[89]. PTH increases sympathetic activity with additional RAAS activation (increase in renin release and aldosterone secretion)^[90] and vascular contractility^[90]. Finally, a cellular interaction through the PTH/PTH-related protein receptor expressed on endothelial cells^[91], VSMC^[92] and inflammatory cells^[93] may directly affect the vascular function.

CLINICAL STUDIES

The association between vitamin D levels and blood pressure was previously reported, observing higher blood pressure trends in the winter months and location further from the equator^[94]. Many clinical studies have subsequently provided consistent results but this topic is still widely debated, especially after the results observed in the interventional clinical trials.

Cross-sectional studies

A large number of cross-sectional studies investigated

the relationship between vitamin D deficiency and blood pressure, as well as the prevalence of hypertension. Table 1 summarizes the studies having 25(OH) vitamin D as reference for the vitamin D status^[1].

The most relevant results were acquired from the national health and nutrition examination survey (NHANES), widely representative of non-hospitalized United States civilian population. First Martins *et al.*^[95] showed an increased prevalence of hypertension associated with low serum 25(OH) vitamin D levels in 15088 subjects from this cohort. In addition, the very large sample size of this cohort allowed to recognize the inverse relationship between 25(OH) vitamin D and raised blood pressure also in several subgroups (such as African Americans and older people^[96,97], children/adolescents^[101,112], Hispanic people^[113], in addition to observed an increased prevalence of pre-hypertension in 25(OH) vitamin D deficient subjects^[121]). Other cross-sectional cohort studies with large sample size supporting these findings were the German National Health Interview and Examination Survey (4030 subjects)^[98], the 1958 British birth cohort (6810 subjects)^[100], and the Tromsø Study (4125 subjects)^[104] as well as the cohorts collected from Israel people (34874 subjects)^[108] and Copenhagen population^[123]. Other smaller cohorts supporting these insights were collected in Europe^[73,103,109,111,122,126], North America^[110,118,120,124], Oceania^[102] and Asia^[72,105,115]. Despite the large numbers of subjects and their worldwide distribution, a clear relationship between vitamin D and blood pressure has not yet been established so far. In fact, among the studies listed in Table 1, seven did not confirm this association^[64,67,70,72,73,119,123]. These conflicting results are in accordance with some unanswered questions in the field of vitamin D biology. In fact, despite the standardization of the season of subject recruitment, the latitudes, where studies were carried out, determine a confounding effect related to the pivotal role of sunlight exposure and consequent vitamin D synthesis within the skin^[2]. Another potential bias is that differences in serum 25(OH) vitamin D levels might depend on the age. Elderly subjects have a reduced skin synthesis and intestinal absorption of vitamin D in addition to spend less time outdoors, limiting sunlight exposure^[127]. Regardless of the latitude and season, only few studies have estimated sun exposure and dietary intake (as well as a possible supplementation) of vitamin D, especially in the elderly population. Moreover, racial differences should be recognized, since the black population correlated with a higher incidence of vitamin D deficiency (and also hypertension), because of their high skin content of melanin^[128]. In this regard, it should be emphasized that most of the negative studies were made up from Caucasian^[67,73,123] Hispanic^[119] and Chinese^[64,72] cohorts. Finally, there is still much debate about which cut-off value defines 25(OH) vitamin D deficiency. However, among the results reported in Table 1 most of the studies showed the first quartile or proposed a cut-off closed to 30 nmol/L. In addition, for higher mean 25(OH) vitamin D levels, blood pressure poorly correlated with vitamin D but rather with PTH

Table 1 Cross-sectional studies evaluating vitamin D blood pressure

Ref.	Year	Study design (sample size)	Country (ethnicity) Age	Correlation (lower reference range of 25(OH) vitamin D)	Findings
Snijder <i>et al</i> ^[67]	2007	Cross-sectional from the LASA (1205 subjects more than 65 yr old)	Netherlands (caucasian) men and women ≥ 65 yr	No (I quartile: < 25 nmol/L)	25(OH) vitamin D was not associated with systolic or diastolic BP or prevalence of hypertension. Instead, PTH correlated with both BP and hypertension incidence
Martins <i>et al</i> ^[95]	2007	Cross-sectional from the 1988-1994 NHANES (15088 subjects)	United States (Caucasian and African Americans and other) men and women age stratified	Yes (I quartile: < 52.5 nmol/L)	Adjusted inter-quartile analysis showed an increased prevalence of hypertension in the lower quartile of 25(OH) vitamin D (OR = 1.30, 95%CI: 1.13-1.49; <i>P</i> < 0.05)
Scragg <i>et al</i> ^[96]	2007	Cross-sectional from the 1988-1994 NHANES (12644 subjects not treated with anti-hypertensive drugs)	United States (Caucasian and African Americans and other) men and women age stratified	Yes (I quintile: < 40 nmol/L)	Adjusted inter-quintile analysis of 25(OH) vitamin D showed significant inverse correlation with both systolic (<i>P</i> < 0.01) and diastolic (<i>P</i> < 0.05) BP. This association was stronger in more than 50 years old and black people
Judd <i>et al</i> ^[97]	2008	Cross-sectional from the 1988-1992 NHANES (7699 non-hypertensive subjects)	United States (White and black people) men and women age stratified	Yes (Vitamin D deficiency defined as < 50 nmol/L)	Lower 25(OH) vitamin D concentrations were associated with a higher blood pressure category in white people (<i>P</i> < 0.01) but after adjustment for age the association was no longer significant
Hintzpeter <i>et al</i> ^[98]	2008	Cross-sectional from GNHIES (4030 adults)	Germany (Caucasian) men and women 18-79 yr	Yes (Vitamin D deficiency defined as < 12 nmol/L ^[99])	According to 25(OH) vitamin D levels, in multivariate analysis there was a relationship between 25(OH) vitamin D and hypertension both in men (OR = 0.97, 95%CI: 0.94-0.99; <i>P</i> < 0.05) and in women (OR 0.96, 95%CI: 0.93-0.99; <i>P</i> < 0.05)
Hypponen <i>et al</i> ^[100]	2008	Cross-sectional from 1958 British birth cohort (6810 subjects)	United Kingdom (Caucasian) men and women 45-47 yr	Yes (I tertile: < 45 nmol/L)	The lower 25(OH) vitamin D tertile was associated with hypertension (OR 0.72, 95%CI: 0.61-0.86; <i>P</i> < 0.01)
Reis <i>et al</i> ^[101]	2009	Cross-sectional from the 2001-2004 NHANES (3577 non-pregnant adolescents without diagnosed diabetes)	United States (Caucasian and African Americans and other) male and female adolescent 12-19 yr	Yes (I quartile: < 37.5 nmol/L)	25(OH) vitamin D was inversely associated with systolic BP (<i>P</i> < 0.05) also in the adjusted odds ratio for the interquartile comparison (OR = 2.36, 95%CI: 1.33-4.19; <i>P</i> < 0.05)
Pasco <i>et al</i> ^[102]	2009	Cross-sectional (861 subjects)	Australia (Caucasian) women: 20-92 yr	Yes (I tertile 25(OH)D: < 30 nmol/L)	In this cohort there was a significant inter-tertile difference in mean BP (<i>P</i> < 0.001) as well as in anti-hypertensive medication use (<i>P</i> < 0.01)
Almirall <i>et al</i> ^[103]	2010	Cross-sectional (237 subjects more than 64 years old)	Spain (Caucasian) men and women 64-93 yr	Yes (cut-off for vitamin D deficiency: < 62.5 nmol/L)	A significant negative association was observed between serum 25(OH) vitamin D levels and both systolic (<i>P</i> < 0.05) and diastolic BP (<i>P</i> < 0.05) also in multivariate analysis
Jorde <i>et al</i> ^[104]	2010	Cross-sectional from the Tromsø Study (4125 subjects not treated with anti-hypertensive drugs)	Norway (Caucasian) Men and women age stratified	Yes (I quartile: < 41.4 nmol/L)	At adjusted inter-quartile analysis serum 25(OH) vitamin D was inversely correlated with systolic BP (<i>P</i> < 0.01)
Kim <i>et al</i> ^[105]	2010	Cross-sectional (1330 subjects)	South Korea (Asian)	Yes (I quintile: < 29.7 nmol/L)	At adjusted inter-quintile analysis, both systolic and diastolic BP decreased linearly with increasing of 25(OH) vitamin D (quintile 1-5; <i>P</i> for trend < 0.01). Moreover, inter-quintile comparison of BP had OR of 0.42 (95%CI: 0.24-0.73; <i>P</i> < 0.05)
Zhao <i>et al</i> ^[106]	2010	Cross-sectional from the 2003-2006 NHANES (5414 subjects not assuming anti-hypertensive drugs)	Men and women < 40 yr United States (Hispanic, Caucasian and African Americans)	Yes (I quintile: < 37.5 nmol/L)	Across 25(OH) vitamin D quintiles systolic and diastolic BP decreased linearly and inversely (<i>P</i> < 0.01). Moreover, the prevalence ratio for hypertension was lower in the highest quintile (OR = 0.82, 95%CI: 0.73-0.91; <i>P</i> < 0.05)
Fraser <i>et al</i> ^[107]	2010	Cross-sectional from the 2001-2006 NHANES (3958 subjects)	United States (Caucasian and African Americans and other) men and women ≥ 20 yr	Yes (linear correlation)	25(OH) vitamin D has an inverse linear correlation with systolic blood pressure in various adjusted models (<i>P</i> < 0.05)

Steinvil <i>et al</i> ^[108]	2011	Cross-sectional case-control study (34874 subjects of which 8387 hypertensive)	Israel men and women 38-72 yr	Yes (vitamin D deficiency defined as < 37.5 nmol/L)	The age-adjusted OR for hypertension among normal and deficient serum 25(OH) vitamin D was 1.19 (95%CI: 1.09-1.31; $P < 0.01$) in women, whereas in men there was not statistical difference
Burgaz <i>et al</i> ^[109]	2011	Cross-sectional from the ULSAM (833 adult men)	Sweden (Caucasian) Men 71 yr	Yes (vitamin D deficiency defined as < 37.5 nmol/L)	Adjusted logistic regression confirmed the association between 25(OH) vitamin D concentration < 37.5 nmol/L and hypertension (OR = 3.3, 95%CI: 1.0-11.0; $P < 0.05$)
Bhandari <i>et al</i> ^[110]	2011	Cross-sectional (2722 subjects of which 1415 hypertensive)	United States (Caucasian and African Americans and other) men and women mean age 58.5 yr	Yes (I quartile: < 37.5 nmol/L)	The prevalence rate of hypertension was inversely correlated with serum 25(OH) vitamin D. Inter-quartile comparison showed an adjusted OR of 2.70 (95%CI: 1.41-5.19; $P < 0.05$)
Pacifico <i>et al</i> ^[111]	2011	Cross-sectional case-control study (452 children and adolescent of which 304 over-weight/obese and 148 normal weight)	Italy (Caucasian) Male and female children	Yes (I tertile of 1,25(OH)2 vitamin D: < 42.5 nmol/L)	1,25(OH)2 vitamin D was inversely correlated with systolic BP both in the whole population ($P < 0.01$) and over-weight ($P < 0.01$) population as well as in control group ($P < 0.01$). Regardless of model for adjusted analysis, the OR for hypertension among tertile categories had a P value < 0.05.
Williams <i>et al</i> ^[112]	2011	Cross-sectional from 2003-2006 NHANES (5617 adolescent)	United States (Caucasian and African Americans and other) male and female children 12-19 yr	Yes (linear correlation)	In this cohort, 25(OH) vitamin D showed a linear inverse association with systolic BP in multivariate analysis ($P < 0.01$).
Forrest <i>et al</i> ^[113]	2011	Cross-sectional from 2005-2006 NHANES (4495 adults subjects of which 1482 hypertensive)	United States (Caucasian and African Americans and other) Men and women age stratified	Yes (vitamin D deficiency defined < 50 nmol/L ^[114])	Vitamin D deficiency independently correlated with prevalence of hypertension ($P < 0.01$).
He <i>et al</i> ^[70]	2011	Cross-sectional from 2003-2006 NHANES (7561 of which 1849 treated with anti-hypertensive drugs)	United States (Caucasian and African Americans and other) Men and women age stratified	No (I quintile: < 33 nmol/L)	25(OH) vitamin D was inversely associated with systolic BP. However, 25(OH) vitamin D lost its statistical significance in a multivariate analysis including PTH. Instead, PTH maintained a strong correlation with BP in multivariate analysis regardless of covariates.
Dorjgochoo <i>et al</i> ^[115]	2012	Cross-sectional study from two, population-based, prospective cohort studies (1460 subjects of which 547 hypertensive)	China (Asian) men and women 40-74 yr	Yes (lowers range defined by I quintile 23.5 nmol/L and cut-offs of 37.5 nmol/L ^[116] and 27.5 nmol/L ^[117])	Among men cohort, BP was inversely and significantly correlated with 25(OH) vitamin D ($P < 0.05$). Moreover, prevalence of hypertension was inversely associated with non-deficient status of vitamin D (adjusted OR = 0.29, 95%CI: 0.10-0.82; $P < 0.05$)
Sakamoto <i>et al</i> ^[118]	2013	Cross-sectional from the AHS-2 (568 subjects)	United States (equally matched Caucasian and African Americans) men and women 30-95 yr	Yes (vitamin D deficiency defined as < 50 nmol/L)	Regardless of adjusted analysis models, Caucasian people showed a linear inverse correlation between 25(OH) vitamin D and BP ($P < 0.05$). Also the comparison between vitamin D deficient and non-deficient showed statistical difference ($P < 0.05$).
Li <i>et al</i> ^[64]	2012	Cross-sectional (1420 subjects of which 487 hypertensive)	China (Asian) Men and women ≥ 65 yr	No (I quartile: < 42 nmol/L)	Serum 25(OH) vitamin D levels were not associated with risk of hypertension in single and multiple regression models. Similarly, PTH is not independently associated with BP or risk of hypertension
Caro <i>et al</i> ^[119]	2012	Cross-sectional (219 subjects of which 115 hypertensive)	Puerto Rico (Hispanic) Men and women 21-50 yr	No (cut-off used to define non optimal: 75 nmol/L)	Vitamin D status was not found to be associated with BP
Chan <i>et al</i> ^[72]	2012	Cross-sectional (939 men aged 65 yr and older)	China (Asian) men ≥ 65 yr (age stratified)	No (I quartile: < 63 nmol/L)	Vitamin D status was not found to be associated with BP. Instead, PTH was directly and independently associated with BP also in multivariate analysis.
Parikh <i>et al</i> ^[120]	2012	Cross-sectional (701 adolescents)	United States (Caucasian and African Americans) Male and female 14-18 yr	Yes (I tertile: < 54.8 nmol/L)	Serum 25(OH) vitamin D has a linear inverse correlation with both systolic ($P < 0.05$) and diastolic ($P < 0.01$) BP. However, in the adjusted analysis only the relationship with systolic BP remained significant.
Sabanayagam <i>et al</i> ^[121]	2012	Cross-sectional from NHANES III (9215 subjects of which 3712 with pre-hypertension)	United States (Caucasian and African Americans and other) men and women age stratified	Yes (I quartile: < 44.25 nmol/L)	In this cohort the systolic BP are inversely correlated with the vitamin D status ($P < 0.05$) and lower values of 25(OH) vitamin D were associated with increase prevalence of pre-hypertension (adjusted OR = 1.48, 95%CI: 1.16-1.90; P value for trend < 0.01).

van Ballegooijen <i>et al</i> ^[122]	2012	Cross-sectional from the Hoorn study (256 subjects)	The Netherlands (Caucasian) men and women 50-75 yr	Yes (I quartile: < 60.8 nmol/L)	In this cohort there was an inverse correlation between 25(OH) vitamin D and both systolic and diastolic BP (<i>P</i> value for trend < 0.01 for both)
Skaaby <i>et al</i> ^[123]	2012	Cross-sectional 4330 subjects)	Denmark (Caucasian) men and women 30-60 yr	No (I quartile: < 33 nmol/L)	Mean 25(OH) vitamin D levels did not differed between hypertensive and normotensive subjects. There was not increased prevalence of hypertension in vitamin D deficient subjects
Kruger <i>et al</i> ^[124]	2013	Cross-sectional form the PURE study (291 African women)	All over the world countries (African) women > 47 yr	Yes (vitamin D deficiency defined as < 30 nmol/L ^[125])	Both systolic and diastolic BP correlated linearly and inversely with serum 25(OH) vitamin D level (<i>P</i> < 0.05 for both). However, only systolic BP maintain statistical significance in multivariate analysis (<i>P</i> < 0.05).
Mateus-Hamdan <i>et al</i> ^[73]	2013	Cross-sectional (284 geriatric patients of which 106 hypertensive)	France men and women mean age 85 ± 6 yr	No (linear correlation)	Means PTH but not 25(OH) vitamin D levels are significant different in hypertensive compared to normotensive patients.
Ke <i>et al</i> ^[126]	2013	Cross-sectional from the ATBC (2271 subjects of which 1430 hypertensive)	Finland (Caucasian) men and women 50-69 yr	Yes (I quartile: < 25 nmol/L)	Serum 25(OH) vitamin D level has a significant and inverse association with systolic BP (<i>P</i> < 0.05), also if stratified in groups. Moreover, the lower group was associated with increased prevalence of hypertension in multivariate analysis (<i>P</i> value for trend < 0.05).

LASA: Longitudinal aging study amsterdam; 25(OH)D: Cholecalciferol; BP: Blood pressure; PTH: Parathyroid hormone; NHANES III: Third United States national health and nutrition examination survey; OR: Odds ratio; GNHIES: German national health Interview and examination survey; ULSAM: Uppsala Longitudinal study of adult men; AHS-2: Adventist health study-2; PURE study: Prospective urban and rural epidemiology study; ATBC: Alpha-tocopherol and beta-carotene study.

levels^[67,70].

Longitudinal studies

Few studies have investigated the incidence of hypertension in vitamin D-deficient subjects. In addition, no study among them had this aim as a primary outcome, suggesting some potential limitation in the statistical power estimation. In addition, the majority of the cohorts investigated was limited to the Caucasian race and female gender, further limiting the generalizability of the results. However, we believe that the main limitation is represented by the lack of prospective risk evaluation in the elderly. In fact, even if the follow-up is extended over 65 years, this overlap does not recognize the critical alterations in vitamin D metabolism during aging. Taking those important limitations into the account, Table 2 summarizes the most important longitudinal observational studies, starting from the results of health professionals' follow-up study (HPFS) and the nurse health study (NHS)2.

Forman *et al*^[129] firstly reported an increased risk of incident hypertension in 1811 subjects selected from these two matched cohorts at 4-year follow-up (pooled RR = 3.18; 95%CI: 1.39-7.29, *P* < 0.05). In addition, the investigators extended this risk prediction, as a surrogate, to the overall study population including 38388 man from HPFS (adjusted RR = 2.31; 95%CI: 2.03-2.63, *P* < 0.05) and 77531 women from the NHS 2 (adjusted RR = 1.57; 95%CI: 1.44-1.72, *P* < 0.05). Afterwards, the same authors also designed a prospective nested case-control study including 1484 normotensive women from the NHS 2 that confirmed the previous results (inter-quartile OR = 1.66; 95%CI: 1.11-2.48, *P* value for trend = 0.01)^[132]. Also the Intermountain Heart Collaborative Study Group provided similar results prospectively ana-

lyzing a large electronic medical database of a general healthcare population. In addition to recognize a wide prevalence of vitamin D deficiency, very low levels of 25(OH) vitamin D were directly associated with an increased risk of developing CV disease, including hypertension (HR = 1.62; 95%CI: 1.48-2.02, *P* < 0.01)^[133]. Significant association between vitamin D deficiency and incidence of hypertension was also observed in a smaller subgroup analysis from both woman cohort of Michigan Bone Health and Metabolism Study (OR = 3.0; 95%CI: 1.01-8.7, *P* < 0.05)^[134] and for male population of Physicians' Health Study (HR = 0.69; 95%CI: 0.50-0.96, *P* < 0.05)^[136]. On the other hand, other large sample size studies such as subgroup analyses from Ely study^[131], Tromsø study (burdened with a 40% dropout rate)^[104], Women's Health Initiative^[135] and Alpha-Tocopherol and Beta-Carotene study cohort^[126], as well as cohort of general Copenhagen population^[123] did not confirm any association between vitamin D levels and incidence of hypertension.

Randomized clinical trials

Table 3 summarizes randomized interventional clinical trials investigating the link between vitamin D and blood pressure.

Although most of the studies reported a significant serum 25(OH) vitamin D increase after supplementation, they are impeded by several limitations, mostly related to study design issues. The first one consists in the limited number of trials investigating blood pressure as a primary outcome. In addition, only few studies focused on vitamin D-deficient cohorts, more suitable for investigating the effectiveness of a supplementation with vitamin D. In this regard, a subgroup analysis of vitamin D-deficient

Table 2 Longitudinal studies addressing the association between vitamin D and blood pressure

Ref.	Year	Study design and follow-up (sample size)	Country (ethnicity) Age	Correlation (lower reference range of 25(OH) vitamin D)	Findings
Forman <i>et al</i> ^[129]	2007	Prospective observational nested case-control study from HPFS and NHS-2 4 yr (1811 subjects)	United States (Caucasian) men 47-82 yr women 43-68 yr	Yes (vitamin D deficiency defined as < 37.5 nmol/L ^[130])	Multivariate RR of incident hypertension among vitamin D deficient subject was 3.18 (95% CI: 1.39-7.29; <i>P</i> < 0.05)
Forouhi <i>et al</i> ^[131]	2008	Prospective observational from the Ely study 10 yr (534 subject)	United Kingdom (Caucasian) men and women 40-69 yr	No (vitamin D deficiency defined as < 25 nmol/L)	There were not significant changes in BP during the follow-up
Forman <i>et al</i> ^[132]	2008	Prospective observational nested case-control study from the NHS 2 7 yr (1484 normotensive women)	United States (Caucasian) women: 32-52 yr	Yes (I quartile: < 21 nmol/L)	Median 25(OH) vitamin D were lower in women developing hypertension (<i>P</i> < 0.01). Moreover, interquartile analysis showed significant and inverse correlation between 25(OH) vitamin D and hypertension (OR = 1.66, 95% CI: 1.11-2.48; <i>P</i> value for trend < 0.05)
Jorde <i>et al</i> ^[104]	2010	Prospective observational from the Tromsø Study 14 yr (4125 subjects not treated with anti-hypertensive drugs)	Norway (Caucasian) men and women 25-84 yr	No (I quartile: < 41.4 nmol/L)	At adjusted analysis, 25(OH) vitamin D did not predict future hypertension or increase in BP: Moreover there was not any association between change in serum 25(OH) vitamin D and BP
Anderson <i>et al</i> ^[133]	2010	Prospective observational average 1.3 yr (maximum 9.1 yr) (41497 subjects)	United States men and women 34-76 yr	Yes (vitamin D deficiency defined as < 37.5 nmol/L)	Lower 25(OH) vitamin D levels were associated with higher incidence of hypertension (HR = 1.62, 95% CI: 1.48-2.02; <i>P</i> < 0.01)
Griffin <i>et al</i> ^[134]	2011	Prospective observational from MBHMS 14 yr (559 women)	United States (Caucasian) women 24-44 yr	Yes (vitamin D deficiency defined as < 80 nmol/L)	25(OH) vitamin D insufficiency has an increased risk of systolic hypertension at multivariate analysis (OR = 3.0, 95% CI: 1.01-8.7; <i>P</i> < 0.05)
Margolis <i>et al</i> ^[135]	2012	Prospective observational from the WHI 7 yr (4863 post-menopausal women)	United States (Caucasian, African, Hispanic, Asian and others) women 50-79 yr	No (I quartile: < 34.4 nmol/L)	There was not significant linear or nonlinear trend in the risk of incident hypertension
Wang <i>et al</i> ^[136]	2012	Prospective observational form PHS 15.3 yr (1211 normotensive men)	United States men 40-84 yr	Yes (I quartile: < 39.9 nmol/L)	There was significant difference only between I and III quartile (HR = 0.69, 95% CI: 0.50-0.96; <i>P</i> < 0.05)
Skaaby <i>et al</i> ^[123]	2012	Prospective observational 5 yr (4330 subjects)	Denmark (Caucasian) men and women 30-61 yr	No (I quartile: < 33 nmol/L)	Multivariate logistic regression analyses did not show any association between 25(OH) vitamin D incidence rate of hypertension.
Ke <i>et al</i> ^[126]	2013	Prospective observational from the ATBC 4 yr (2271 subjects of which 1430 hypertensive)	Finland (Caucasian) men and women 50-69 yr	No (I quartile: < 25 nmol/L)	25(OH) vitamin D did not predict future hypertension.

HPFS: Health professionals' follow-up study; NHS 2: Nurse health study 2; 25(OH)D: Cholecalciferol; RR: Relative risk; BP: Blood pressure; OR: Odds ratio; HR: Hazard ratio; MBHMS: Michigan bone health and metabolism study; WHI: Women's health initiative; PHS: Physicians' health study; ATBC: Alpha-tocopherol and beta-carotene study cohort.

subjects, from a sample of 112 Danish hypertensive patients randomized to high-dose 25(OH) vitamin D supplementation (75 µg/d) versus placebo, showed a significant decrease of 24-h systolic and diastolic blood pressure values (*P* < 0.05)^[155]. These findings confirmed previous results from other small sample size cohorts of vitamin D-deficient patients^[141,142,150]. For this reason, the recently results by Forman *et al*^[157] from the largest published cohort of hypertensive patients (*n* = 283) randomized to vitamin D supplementation versus placebo appear of particular interest. The oral administration of 25(OH) vitamin D (25 to 100 µg/d) significantly decreased the

blood pressure levels. Unfortunately, these studies present additional limitations, such as taking into account the different approaches used for vitamin D supplementation. Although sunlight exposition might be the more physiological way, the ultraviolet (UV)-B rays-induced skin synthesis of vitamin D is hard to quantify and thus poorly investigated^[140,151]. Oral supplementation has been preferred because easier to manage (despite some variability in intestinal absorption may exist) if provided through diet regimen^[147], nutritional supplements^[146] or direct vitamin D administration (daily intake^[137-139,141,143,144,152-157] or loading dose^[142,148-150,158]). Finally, it should be reported

Table 3 Randomized clinical trial investigating the protective effect of vitamin D supplementation on blood pressure

Ref.	Year	Study design	Country (ethnicity) Age	Intervention	Findings
Lint <i>et al</i> ^[137]	1988	(sample size) Prospective randomized double-blind placebo-controlled trial (65 men with glucose intolerance of which 26 hypertensive)	Sweden (Caucasian) 61-65 yr	(follow-up) α -calcidol 0.75 μ g (12 wk)	In hypertensive patients supplementation has additive effect to concomitant antihypertensive therapy in reducing BP ($P < 0.01$). In the whole population there was only non-significant trend in BP lowering
Pan <i>et al</i> ^[138]	1993	Prospective randomized double-blind 2 \times 2 interventional trial (58 institutionalized elderly persons)	Taiwan (Asian) not provided	calcium 800 mg/d or 1,25(OH) ₂ vitamin D 5 μ g/d or calcium 800 mg/d + 1,25(OH) ₂ vitamin D 5 μ g/d, or placebo (11 wk)	Any type of supplementation failed to reduce BP
Scragg <i>et al</i> ^[139]	1995	Prospective randomized double-blind placebo-controlled trial (189 elderly subjects)	United Kingdom (not provided) 63-76 yr	25(OH) vitamin D 2.5 μ g/d or placebo (5 wk)	Although treatment was effective in increasing serum 1,25(OH) ₂ vitamin D ($P < 0.01$) and decreasing PTH ($P < 0.01$), there was not difference in BP change
Krause <i>et al</i> ^[140]	1998	Prospective randomized double-blind controlled trial (18 patients with untreated mild essential hypertension)	Germany (Caucasian) 26-66 yr	Full body UVB or UVA thrice weekly (6 wk)	In accordance with a 162% rise in plasmatic 25(OH) vitamin D ($P < 0.01$) and 15% fall in serum PTH ($P < 0.01$), the UVB group showed also a reduction in 24-h ambulatory systolic and diastolic BP ($P < 0.01$)
Pfeifer <i>et al</i> ^[141]	2001	Prospective randomized double-blind controlled trial (148 elderly subject with 25(OH)D < 50 nmol/L)	Germany (Caucasian) 70-86 yr	Calcium 600 mg \times 2/d or calcium 600 mg + 25(OH) vitamin D 10 μ g twice daily (8 wk)	In accordance with a 72% rise in plasmatic 25(OH) vitamin D ($P < 0.01$) and 17% fall in serum PTH ($P < 0.05$), combined supplementation significantly reduced systolic BP ($P < 0.05$)
Sudgen <i>et al</i> ^[142]	2008	Prospective randomized double-blind placebo-controlled trial (34 elderly type 2 diabetic patients with 25(OH)D < 50 nmol/L)	United Kingdom (not provided)	Loading dose ergocalciferol 2500 μ g or placebo (8 wk)	Supplementation significantly rise plasmatic 25(OH) vitamin D ($P < 0.01$) and reduced systolic BP, whereas there was only a trend in diastolic BP decrease
Alborzi <i>et al</i> ^[143]	2008	Prospective randomized double-blind placebo-controlled trial (24 elderly type 2 diabetic patients with 25(OH)D < 50 nmol/L)	mean 64 years United States (Caucasian and African Americans) 56-80 yr	Paricalcitol 1 or 2 μ g/d or placebo (4 wk)	Any dose of paricalcitol failed to reduce BP
Margolis <i>et al</i> ^[144]	2008	Prospective randomized double-blind controlled trial (36282 n post-menopausal women from WHI study)	United States (Caucasian, Asian, Hispanic, African American) 50-79 yr	Calcium 500 mg \times 2/d or calcium 500 mg + 25(OH) vitamin D 5 μ g twice daily (7 yr)	There was no significant difference in over time change of BP in the whole population. In addition, supplementation failed to reduce the risk of developing hypertension in non-hypertensive patients at baseline
Nagpal <i>et al</i> ^[145]	2008	Prospective randomized double-blind placebo-controlled trial (71 older overweight men)	India (Indian population) 36-54 yr	25(OH) vitamin D 3000 μ g every 2 wk for 3 times or placebo (7 wk)	Supplementation failed to reduce BP
Daly <i>et al</i> ^[146]	2009	Prospective randomized double-blind controlled trial (124 community-dwelling men)	Australia (Caucasian) 55-69 yr	Milk fortified with calcium (500 mg) and 25(OH) vitamin D (10 μ g) twice a day or standard milk (2 yr)	Supplementation failed to reduce BP
Hilpert <i>et al</i> ^[147]	2009	Prospective randomized double-blind controlled trial (23 hypertensive adults)	United States (not provided)	Dairy-rich, high fruits and vegetables diet or a high fruits and vegetables diet or an average Western diet (5 wk)	High fruits and vegetables diet dairy-rich or not significantly reduced BP ($P < 0.05$). Moreover, in dairy-rich, high fruits and vegetables diet there was a greater lowering of intracellular calcium ($P < 0.01$), strongly associated with fall in diastolic BP ($P < 0.05$)
Witham <i>et al</i> ^[148]	2010	Prospective randomized double-blind placebo-controlled trial (56 patients with history of stroke and baseline 25(OH)D < 75 nmol/L)	United Kingdom (not provided) 53-79 yr	Loading dose ergocalciferol 2500 μ g or placebo (8 and 16 wk)	Supplementation significantly increased serum 25(OH) vitamin D to both controls ($P < 0.01$). However, treatment failed to reduced BP

Witham <i>et al</i> ^[149]	2010	Prospective randomized double-blind placebo-controlled trial (61 patients with type 2 diabetes and baseline 25(OH)D < 100 nmol/L)	United Kingdom (not provided) 55-76 yr	Loading dose ergocalciferol 2500 µg or 5000 µg or placebo (8 and 16 wk)	Supplementation significantly increased serum 25(OH) vitamin D to both controls ($P < 0.01$ for both). However, supplementation failed to reduced BP
Judd <i>et al</i> ^[150]	2010	Prospective randomized double-blind controlled trial (9 patients with baseline 25(OH)D within 25 and 75 nmol/L in addition to systolic BP between 130 and 150 mmHg)	United States (African American) mean 45 yr	loading dose ergocalciferol 2500 µg or placebo weekly for 3 wk or 25 (OH) vitamin D 0.5 µg twice a day for 1 wk (3 wk)	Only supplementation with 25(OH) vitamin D decrease by 9% mean systolic BP ($P < 0.01$) in accordance with rise of serum 25(OH) vitamin D ($P < 0.05$)
Scragg <i>et al</i> ^[151]	2011	Prospective randomized double-blind controlled trial (119 patients with baseline 25(OH)D < 50 nmol)	New Zealand (Pacific islander, Caucasian and Maori) 23-87 yr	24 whole body exposures of either UVB or ultraviolet A (6 and 12 wk)	In the UVB arm there was a significant increase in serum 25 (OH) vitamin D after both 6 and 12 wk ($P < 0.01$ for both). However, treatment failed to reduced BP
Salehpour <i>et al</i> ^[152]	2012	Prospective randomized double-blind placebo-controlled trial (77 pre-menopausal overweight and obese women)	Iran (Arabian) 30-46 yr	25 (OH) vitamin D 25 µg daily or placebo (12 wk)	Supplementation significantly rise plasmatic 25 (OH) vitamin D ($P < 0.01$) and fall PTH ($P < 0.01$). Moreover, although treatment improved lipid profile, there was no effect on BP
Gepner <i>et al</i> ^[153]	2012	Prospective randomized double-blind placebo-controlled trial (110 post-menopausal women with baseline 25(OH)D within 10 and 60 nmol/L)	United States (not provided) 60-67 yr	25 (OH) vitamin D 62.5 µg daily or placebo (16 wk)	Supplementation, although significantly raised serum 25(OH) vitamin D ($P < 0.01$), failed in improving BP control assessed by changes in FMD, PWV and Aix
Wood <i>et al</i> ^[154]	2012	Prospective randomized double-blind placebo-controlled trial (305 healthy post-menopausal women)	United Kingdom (not provided) 48-72 yr	25 (OH) vitamin D 10 µg or 25 µg/d or placebo (1 yr)	Supplementation failed in improving CV risk profile, including BP control
Larsen <i>et al</i> ^[155]	2012	Prospective randomized double-blind placebo-controlled trial (112 hypertensive patients)	Denmark (Caucasian) 48-72 yr	25 (OH) vitamin D 75 µg/d or placebo (20 wk)	Supplementation significantly rise plasmatic 25 (OH) vitamin D ($P < 0.01$) and fall PTH ($P < 0.01$) but failed in improving BP control. However, in a post-hoc subgroup analysis of patient with 25 (OH) vitamin D deficiency at baseline supplementation significantly decrease 24-h systolic and diastolic BP ($P < 0.05$)
Zhu <i>et al</i> ^[156]	2013	Prospective randomized double-blind placebo-controlled trial (43 healthy subjects)	China (Asian) 20-22 yr	Calcium 600 mg + 25 (OH) vitamin D 3.12 µg daily or placebo, in addition to 500 kcal/d of caloric deficit (7 yr)	Except a reduction in visceral fat mass, supplementation failed in improving CV risk profile, including BP control
Forman <i>et al</i> ^[157]	2013	Prospective randomized double-blind placebo-controlled trial (283 healthy black subjects)	United States (African American) mean 51 yr	25 (OH) vitamin D 25 µg or 50 or 100 µg/d or placebo (12 and 24 wk)	Supplementation significantly decrease BP consistent with increasing dose ($P < 0.05$). Moreover, there was linear correlation between systolic BP decrease and rise of serum 25 (OH) vitamin D ($P < 0.05$)
Witham <i>et al</i> ^[158]	2013	Prospective randomized double-blind placebo-controlled trial (159 with isolate systolic hypertension)	United States (not provided) mean 77 yr	Loading dose 25 (OH) vitamin D 2500 µg or placebo (12, 24 and 36 wk)	Supplementation significantly rise plasmatic 25 (OH) vitamin D ($P < 0.01$) but failed in improving BP control. Moreover, treatment failed to achieve secondary outcomes including 24-h blood pressure, arterial stiffness and endothelial function

α -calcidol: Synthetic analog of 1,25(OH)₂D; BP: Blood pressure; 1,25(OH)₂D: Calcitriol; UVB: 94.5% UVA and 3.5% UVB; UVA: 99.5% UVA and 0.05% UVB; 25(OH)D: Cholecalciferol; PTH: Parathyroid hormone; WHI: Women's Health Initiative Calcium/vitamin D trial; HyD: 25(OH)D metabolite with hydrophilic properties and much shorter half-life; FMD: Brachial artery flow-mediated vasodilation; PWV: Carotid-femoral pulse wave velocity; Aix: Aortic augmentation index; CV: Cardiovascular.

the failure of Women's Health Initiative study to prove changes in blood pressure in a very large sample size of post-menopausal women ($n = 36282$) randomized to receive calcium versus calcium plus 25(OH)D over 7-year follow-up^[144].

Meta-analyses of clinical studies

Five meta-analyses were recently performed to quantify the prospective associations of vitamin D status with the

risk of hypertension. Pittas *et al*^[159] included the results of four observational longitudinal cohorts with 32181 subjects with a follow-up of 7 to 10 years. The pooled analysis showed an increased risk of developing hypertension in vitamin D-deficient subjects (RR = 1.76; 95%CI: 1.27-2.44, $P < 0.05$). Conversely, another meta-analysis of ten randomized clinical trials failed to prove the effectiveness of vitamin D supplementation in promoting blood pressure decrease^[159]. Therefore, this mismatch between

observational studies and randomized interventional clinical trials is retrieved in other meta-analyses. The lack of relationship in interventional studies was reported by Witham *et al.*^[160] and Wu *et al.*^[161], while pooled analysis of observational studies showed a strong association between vitamin D status and blood pressure^[162]. In particular, the meta-analysis of observational longitudinal studies by Kunutsor *et al.*^[163] recently reported that subjects in the higher tertiles of vitamin D levels have a 30% lower risk of developing hypertension as compared to those in the bottom tertiles (pooled RR = 0.70; 95%CI: 0.58-0.86, $P < 0.05$).

OPEN ISSUES AND PERSPECTIVES

Many questions recently emerged from efficacy and safety in interventional trials using vitamin D supplementation. In experimental mouse models, excessive intake of vitamin D induces vascular and soft-tissue calcifications. Thus, in human beings, caution has to be used on the pro-calcifying effects of exogenous vitamin D. In addition to derangement in calcium homeostasis, it should take into account the detrimental effects of vitamin D-induced phosphate overload involving also FGF23/klotho axis. On the other hand, the definition of the optimal vitamin D status from a CV point of view remains matter of debate and general consensus is still missing. “Bone health-driven” recommendations agree to define insufficient a 25(OH) vitamin D levels < 20 ng/mL, suggesting a target of 30 ng/mL. Similarly, reports from large cohorts (such as NHANES^[164] and The Framingham offspring study^[165]) showed a linear inverse association with CV outcome for 25(OH) vitamin D levels up to 30ng/mL. Considering hypertension, the results from the Vitamin D and Omega-3 Hypertension Trial (VITAL Hypertension) that is still enrolling patients^[166] might clarify this point. Finally, the “local vitamin D system” is emerging as a pivotal topic that might explain the conflicting results between observational and interventional trials^[167].

CONCLUSION

Neither the European society of Cardiology nor American Heart Association have published CV-focused algorithms regarding vitamin D deficiency and this is because the first results from randomized clinical trials have provided more questions than answers. Certainly, several factors involved in vitamin D biology are under-recognized or hard to assess, including physical activity, sunlight exposure, health status or dietary habits. Moreover, several confounding factors have not been considered in several studies, such as comorbidities, concomitant medications or differences in gender, age and race. In addition, also vitamin D compounds proposed were highly variable, ranging from native (cholecalciferol or ergocalciferol) or synthetic (α -calcidol) inactive vitamin D to active vitamin D (calcitriol) up to selective VDR activators (paricalcitol). However, it is likely that other unidentified factors are

also involved in vitamin D biology, such as the possible relationship with other endocrine networks, emphasizing the need of pre-clinical studies.

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WJC 6th Anniversary Special Issues (1): Hypertension

Device-guided breathing exercises for the treatment of hypertension: An overview

Kornelis JJ van Hateren, Gijs WD Landman, Susan JJ Logtenberg, Henk JG Bilo, Nanne Kleefstra

Kornelis JJ van Hateren, Gijs WD Landman, Susan JJ Logtenberg, Henk JG Bilo, Nanne Kleefstra, Diabetes Centre, Isala, 8000 GK Zwolle, The Netherlands

Susan JJ Logtenberg, Henk JG Bilo, Nanne Kleefstra, University of Groningen, University Medical Center Groningen, Department of Internal Medicine, 9700RB Groningen, The Netherlands

Henk JG Bilo, Department of Internal Medicine, Isala, 8000GK Zwolle, The Netherlands

Nanne Kleefstra, Langerhans Medical Research Group, 8000GK Zwolle, The Netherlands

Author contributions: van Hateren KJ and Landman GW searched literature; all authors participated in interpretation of the data and revision of the manuscript; van Hateren KJ drafted the manuscript; Bilo HJ and Kleefstra N supervised the study.

Correspondence to: Kornelis JJ van Hateren, MD, Diabetes Centre, Isala, PO Box 10400, 8000 GK Zwolle, The Netherlands. k.j.j.van.hateren@isala.nl

Telephone: +31-38-4242518 Fax: +31-38-4243367

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cial effects on blood pressure. There is only 1 study that used a sham device as a control group. All other studies were to some extent methodologically flawed. Based on the studies with an acceptable methodological quality, there is no clear evidence supporting a short-term beneficial effect on blood pressure by using device-guided breathing.

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Key words: Hypertension; Device-guided breathing; Review

Core tip: This review discusses all randomized controlled trials that have investigated the effects of device-guided breathing on blood pressure. There were 6 studies with an acceptable control group. Two (manufacturer sponsored) trials showed beneficial effects of device-guided breathing, both used listening to music as a control group. The remaining 4 studies observed no beneficial effects. We conclude that there is no sufficient evidence for recommending device-guided breathing in the treatment of hypertension.

Abstract

The American Heart Association considers device-guided breathing as a reasonable treatment modality in their statement on non-pharmacological options for lowering blood pressure. This review discusses all randomized controlled trials that have investigated the effects of device-guided breathing on blood pressure in patients with hypertension. Thirteen studies were included in this review. In total, 627 patients were included, of which 365 patients were allocated to device-guided breathing. Only 6 studies used acceptable control groups: listening to music, meditative relaxation exercises, or a sham-device. Two sponsored trials showed beneficial effects of device-guided breathing, both used listening to music as a control group. The remaining 4 studies, which had no employees of the manufacturer listed as co-author, observed no benefi-

van Hateren KJ, Landman GW, Logtenberg SJ, Bilo HJ, Kleefstra N. Device-guided breathing exercises for the treatment of hypertension: An overview. *World J Cardiol* 2014; 6(5): 277-282 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i5/277.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i5.277>

INTRODUCTION

Treatment of hypertension includes both pharmacological and non-pharmacological interventions. Accepted non-pharmacological interventions are sodium restriction, losing weight, increasing physical activity, smoking cessation and optimizing alcohol consumption^[1-3]. In a scientific statement from the American Heart Associa-

tion (AHA) regarding non-pharmacological options for lowering blood pressure, device-guided slow breathing is described as a reasonable treatment modality to reduce blood pressure (Class II A, Level of Evidence B)^[4]. Device-guided slow breathing aims at lowering the respiratory frequency into a so-called “therapeutic breathing zone” (less than 10 breaths per minute) through biofeedback by using an electronic device. Exercises are regarded as successful if the total exercise time is at least 45 min per week, preferably 15 min daily^[4]. Sympathetic overactivity is hypothesized as an important contributing factor in the development of hypertension^[5-7]. Efforts aimed at reducing this autonomic imbalance may indeed be an effective therapy for hypertension. Slow and regular breathing, guided by musical tones, will lead to a reduction of sympathetic activity and also to an increase in heart rate variability^[5]. The baroreceptors measure blood pressure in the carotid arteries and the aorta, and an increase in pressure leads to parasympathetic activation and vice versa (negative feedback mechanism). As an increase in heart rate variability will lead to an increased baroreflex sensitivity^[5], device-guided breathing may lead to lower blood pressure values.

The conclusions of the writing group of the AHA statement were based on a meta-analysis^[8] and several other studies^[9-19]. After the publication of the guideline, two additional studies have been published^[20,21]. The overall effect estimate in the meta-analysis showed a small beneficial blood pressure lowering effect [a reduction of 3.7 mmHg in systolic blood pressure (SBP)], but the authors of the meta-analysis stated that the results of the overall effect estimates should be interpreted with caution because of methodological flaws in most studies. Beneficial effects were not observed after excluding studies with high risk of bias or studies that were sponsored by or involved the manufacturer of the device^[8]. A previous editorial already emphasized that an independent double-blind study with a proper control group, preferably a sham device, would be necessary to answer the question whether device-guided breathing has any effect on blood pressure^[22]. Recently, an investigator-initiated double-blind and sham-controlled trial was performed^[20]. This review discusses all randomized controlled trials (RCTs) that have investigated the effects of device-guided breathing on blood pressure in patients with hypertension.

PREVIOUS STUDIES

Thirteen studies, of which the study and patient characteristics are presented in Table 1, were included in this review. In total, 627 patients were included, of which 365 patients were allocated to device-guided breathing. Except for 1 study in which a bi-level positive pressure device (BiPAP®) was used^[19], all other studies used the Resperate® device. The Resperate® device uses a form of biofeedback with “breathe in” and “breathe out” instructions according to the listeners breathing rate to guide the respiration into a lower frequency by prolong-

ing expiration. The BiPAP® device was used for the treatment of patients with obstructive sleep apnea and it was also capable of guiding patients’ respiratory rate to less than 10 breaths per minute. Three studies had no control group^[11,12,19], 4 studies compared the intervention to usual care or frequent blood pressure measurements^[13,14,17,21], 4 studies compared the intervention to listening to music^[9,10,15,16], 1 study compared the intervention to meditative relaxation exercises^[18], and 1 study used a sham-device in the control group^[20]. Except for 3 studies^[15,16,20], all other studies were sponsored by or involved the manufacturer of the Resperate® and BiPAP® devices. According to the meta-analysis by Mahtani *et al*^[8], the Anderson paper was also not sponsored by the manufacturer^[18]. However, the acknowledgements section of this manuscript states that Drs. B. Gavish, an employee of the company that manufactures the Resperate® device, had reviewed the paper.

EFFECTS OF DEVICE-GUIDED BREATHING

Table 1 presents an overview of the effects of device-guided breathing on blood pressure. Only 4 studies reported between-group-differences including the 95% confidence intervals^[9,15,16,20]. Significant decreases in blood pressure were observed in all 3 studies without a control group^[11,12,19]. A significant between-group-difference was observed in 2 out of 4 studies that compared device-guided breathing to daily blood pressure measurements^[13], and usual care^[17]. Studies comparing device-guided breathing to usual care cannot differentiate the 3 possible mechanisms through which the Resperate® could have a blood pressure lowering effect: (1) effects of guided slowing of breathing itself; (2) listening to music; and (3) sitting still. Conclusions regarding the isolated effect of device-guided breathing are only valid when a study has an appropriate control group to disentangle these 3 effects. Therefore, this review will further focus on the 6 studies that used acceptable control groups: listening to music, meditative relaxation exercises and a sham-device^[9,10,15,16,18,20]. Two sponsored trials showed beneficial effects of device-guided breathing, both used listening to music as a control group^[9,10]. In the study by Schein *et al*^[9] device-guided breathing was not effective in lowering SBP compared to the control group. This study pre-defined a 5 mmHg reduction in diastolic blood pressure (DBP) as clinically relevant. The difference in DBP change between both groups was 4.4 mmHg in favour of the intervention group ($P = 0.008$). Although a second study failed to predefine a clinically relevant difference, it showed a significant decrease in office SBP compared to a Walkman group (between-group-difference 4.6 mm Hg, $P = 0.001$)^[10]. The remaining 4 studies, which had no employees of the manufacturer listed as co-author, observed no beneficial effects on blood pressure^[15,16,18,20]. Only the study by Landman *et al*^[20] described the presence of 2 negative side-effects, but this was insufficient to conclude

Table 1 Study and patients characteristics

Ref.	Study group		Period (wk)	Study arm		Endpoint	Results (mean)		
	Disease, therapy, patients	Number (I/C)		Intervention	Control		Intervention (mmHg)	Control (mmHg)	Difference intervention vs control (mmHg)
Schein <i>et al</i> ^[9] , 2001; Israel	HT, medication, BP \geq 140/90, 25-75 yr	32/33	8	Resperate® 10 min/d	Walkman 10 min/d	SBP	156.6 > 141.4	154.7 > 143.4	-2.9 (-2.8-10.6)
						DBP	96.7 > 86.7 ^a	93.4 > 87.8	-4.4 ^a (1.1-7.6)
						Clinic, SBP	160 > 152.5	155 > 152.1	-4.6 ^a
						DBP	95 > 91	94 > 92.5	-2.5
Grossman <i>et al</i> ^[10] , 2001; Israel	HT, medication, BP \geq 140/90, 25-75 yr	18/15	8	Resperate® 10 min/d	Walkman 10 min/d	Home, SBP	157 > 152.0	151 > 149.8	-3.8
						DBP	94 > 91.3	90 > 90.9	-3.6 ^a
						24 h, SBP	137.1 > 129.9 ^a	-	-
						DBP	82.5 > 80.2	-	-
Rosenthal <i>et al</i> ^[11] , 2001; Israel	HT, medication, BP 130/85-180/110, 25-75 yr	13/-	8	Resperate® 15 min/d	-	SBP	137.1 > 129.9 ^a	-	-
						DBP	82.5 > 80.2	-	-
Viskoper <i>et al</i> ^[12] , 2003; Israel	HT, medication, SBP 140-160 or DBP 90-100, 40-80 yr	17/-	8	Resperate® 15 min/d	-	Home, SBP	156.4 > 150.0 ^a	-	-
						DBP	88.5 > 85.9 ^a	-	-
						Clinic, SBP	155.4 > 142.5 ^a	-	-
						DBP	88.9 > 82.0 ^a	-	-
Meles <i>et al</i> ^[13] , 2004; Italy	HT, 40-75 yr + 1) not treated, SBP 140-159 or DBP 90-99; OR = 2) medication and BP > 140/90	48/31	8	Resperate® 15 min/d	BP 1/d	Home, SBP	137 > 131.6 ^a	126 > 124.1	-3.5 ^a
						DBP	83 > 79.8 ^a	79 > 78.0	-2.2 ^a
						Clinic, SBP	141.4 > 135.9	133.2 > 133.0	-5.3
						DBP	88.1 > 84.5 ^a	85.9 > 86.8	-4.5 ^a
Elliot <i>et al</i> ^[14] , 2004; United States	HT, medication, SBP 140-179, DBP < 110, 40-75 yr	89/60	8	Resperate® 15 min/d	BP 3/d	Clinic, SBP	150.3 > 139.7	149.8 > 140.6	-1.4
						DBP	84.7 > 81.5	86.8 > 83.6	0.0
						Home, SBP	145.8 > 145.3	141.3 > 141.9	-1.1
						DBP	85.9 > 85.3	83.7 > 83.5	-0.4
Logtenberg <i>et al</i> ^[15] , 2007; The Netherlands	T2DM, HT, medication, SBP 140-160, > 18 yr	15/15	8	Resperate® 15 min/d	Discman 15 min/d	Clinic, SBP	153.5 > 146.0	150.4 > 138.2	4.7 (-11.7-2.3)
						DBP	83.0 > 82.0	87.0 > 81.5	4.6 (-10.4-2.3)
						Home, SBP	-	-	1.0 (-7.8-5.8)
						DBP	-	-	1.3 (-5.8-3.2)
Altena <i>et al</i> ^[16] , 2008; The Netherlands	HT, medication SBP 140-160, > 18 yr	15/15	8	Resperate® 15 min/d	Discman 15 min/d	Clinic, SBP	-9.8	-5.6	-4.2 (-12.4-3.9)
						DBP	-4.6	-2.0	-2.6 (-8.4-3.3)
						Home, SBP	-2.5	-2.9	0.5 (-3.7-4.8)
						DBP	-4.9	-3.4	-1.8 (-8.4-4.8)
Schein <i>et al</i> ^[17] , 2009; Israel	T2DM, HT, medication, SBP > 130	33/33	8	Resperate® 15 min/d	Usual care	Clinic, SBP	150 > 140	147 > 149	-12 ^a
						DBP	81 > 77	81 > 80	-3
						Clinic, SBP	141.8 > ?	140.1 > ?	-
						DBP	88.2 > ?	85.2 > ?	-
Anderson <i>et al</i> ^[18] , 2010; United States	Stage 1 HT or pre-hypertension, no medication, no CVD or T2DM.	20/20	4	Resperate® 15 min/d	Meditative exercise 15 min/d	24h, SBP	138.2 > 137.7	137.3 > 137.8	-1
						DBP	84.6 > 83.8	80.4 > 81.8	-2.2
						Clinic, SBP	140 > 130.4 ^a	-	-
						DBP	82.7 > 80.2	-	-
Bertisch <i>et al</i> ^[19] , 2011; United States	HT and OSA, medication or untreated, BP 120/80-160/100, 20-75 yr	25/-	8	BiPAP® 15 min/d	-	Clinic, SBP	140 > 130.4 ^a	-	-
						DBP	82.7 > 80.2	-	-
						Clinic, SBP	151.6 > 145.6	151.2 > 142.8	2.4 (-6.5-11.2)
						DBP	82.1 > 76.2	80.7 > 77.0	-2.3 (-6.7-2.2)
Landman <i>et al</i> ^[20] , 2013; The Netherlands	T2DM, HT, medication, SBP 140-160, \geq 18 yr	24/24	8	Resperate® 15 min/d	Sham-Device 15 min/d	Home, SBP	?	?	-3.0 (-13.2-7.2)
						DBP	?	?	0.1 (-6.9-7.1)
						24h, SBP	126.1 > 123.2 ^a	?	?
						DBP	?	?	?
Howorka <i>et al</i> ^[21] , 2013; Austria	T2DM, HT, medication, BP < target value, 18-78 yr	16/16	8	Resperate® 12 min/d	Usual care	Daytime SBP	129.3 > 127.1	?	?
						DBP	?	?	?
						Daytime SBP	129.3 > 127.1	?	?
						DBP	?	?	?

^a $P < 0.05$ vs control. I: Intervention; C: Control; HT: Hypertension; SBP: (Systolic) blood pressure; DBP: Diastolic blood pressure; T2DM: Type 2 diabetes mellitus; CVD: Cardiovascular disease; OSA: Obstructive sleep apnea.

that there was a causal relationship with device-guided breathing.

METHODOLOGICAL QUALITY

In order to compare the studies, we assessed the methodological quality using the criteria as described by van Tulder *et al*^[23] (Table 2). The quality of the study by An-

derson *et al.* was low; they used an open randomisation procedure without any further explanation regarding this procedure and blinding^[18]. After carefully evaluating the studies by Schein *et al*^[9] and Grossman *et al*^[10] several methodological questions remained unanswered. It was stated in the Schein *et al*^[9] study that the study had a double-blind study design^[9]. Randomisation was performed by a third party and a special technician delivered and

Table 2 Randomized controlled trials with an active control group: methodological quality

Criteria	Schein ^[9]	Grossman ^[10]	Logtenberg ^[15]	Altena ^[16]	Anderson ^[18]	Landman ^[20]
Randomization adequate	+/-	+	+	+	-	+
Treatment allocation concealed	+	?	+	+	-	+
Groups similar at baseline	+	+	+	+	+	+
Patient blinded	+/-	+/-	+	+	-	+
Care provider blinded	+/-	+/-	-	-	-	+
Outcome assessor blinded	-	?	-	-	-	+
Co-interventions avoided	+	+/-	?	+	?	+
Compliance acceptable	+	?	+	+	+	+
Withdrawal/drop-out rate acceptable	+	+	+	+	+	+
Timing of outcome assessment similar	+	+	+	+	+	+
Intention to treat analyses	+/-	+	+	+	-	+

explained the device and study procedures. Although the doctor was not aware of the group assignment, patients had weekly follow-up meetings including blood pressure measurements by that same person. Patients were requested not to talk about the specific device with their doctor or to other persons who may be participating in the study. As the patients saw their doctor very regularly it is not unlikely that the doctor became aware of group assignment. Therefore, from a methodological point of view, the authors could have opted for another person performing the outcome measurements. An alternative method would have been to check the success of the blinding procedure. The authors did not explain their rationale behind this randomisation procedure. Furthermore, there were several primary endpoints instead of 1 primary endpoint and 2 secondary endpoints. Also, 5% of all blood pressure data were excluded in an unconventional and post-hoc defined 'end of treatment period' analysis.

Grossman *et al*^[10] did not describe whether treatment allocation was concealed and who performed the outcome measurements. Also, data on compliance and whether the blinding procedure was a success, were not provided. Two patients in the control group started lifestyle modification programmes, but analyses without these patients did not change the results.

The Logtenberg, Altena *et al*^[16] and Landman *et al*^[20] studies have one important limitation in common: the width of the 95%CI of the change of office-measured SBP between groups^[15,16,20]. These studies were powered to detect an absolute reduction of 10 mmHg in SBP. In all these studies the limits of the confidence intervals exceeded the boundary of 10 mmHg. The 95%CI in the Logtenberg *et al*^[15] and Landman *et al*^[20] studies ranged from -2.3 mmHg to 11.7 mmHg, and -6.5 mmHg to 11.2 mmHg, respectively, with a direction in favour of the control group^[15,20]. This means that clinically relevant disadvantageous effects of device-guided breathing could not be ruled out. For the Altena *et al*^[16] study, the confidence interval ranged from -12.4 mmHg to 3.9 mmHg with a direction in favour of the intervention group^[16]. Logtenberg *et al*^[15] did not provide data on avoiding co-interventions, whereas Altena *et al*^[16] reported that 1 patient in the control group had a change in antihypertensive therapy (per-protocol analyses showing the same results).

HbA1c level was higher in the intervention group of the Landman *et al*^[20] study, but additional analyses in which adjustments for age, gender, body mass index and HbA1c were done did not relevantly change the results^[20]. The adjusted differences in SBP and DBP were 1.1 mmHg (95%CI: -7.6-9.8, in favour of the control group) and 3.5 mmHg (95%CI: -0.4-7.4, in favour of the intervention group), respectively. Finally, the Logtenberg *et al*^[15] and Altena *et al*^[16] studies had a single-blind design.

Sample size calculations were described in 4 studies^[9,15,16,20], and lacking in the Anderson *et al*^[18] and Grossman *et al*^[10] studies^[10,18]. Although Grossman *et al*^[10] mentioned that the group size was large enough, they didn't provide a calculation^[10]. The Logtenberg study based the calculation on mean SBP and standard deviation (SD) in their clinic^[15]. Altena *et al*^[16] used the mean blood pressure and SD that were observed in the Logtenberg *et al*^[15] study. The most conservative and optimal calculation was performed in the Landman study, as they based their sample size on the highest SD of the change in SBP in the Logtenberg *et al*^[15] (SD 9.4 mmHg) and Altena *et al*^[16] (SD 10.9 mmHg) studies^[20]. Comparable to their data analysis, Schein *et al*^[9] used an unconventional method for the estimation of their sample size. The standardised detectable difference was based on a previous study^[24] while they could have used the change in blood pressure and its SD.

DISCUSSION

Out of the 13 RCTs published, there were only a few studies with an acceptable methodological quality. All studies had a short follow-up period. In order to exert effects on cardiovascular morbidity by using device-guided breathing, the device has to be used for many months and preferably years. None of the studies investigated whether the device could be used for prolonged periods. There is 1 meta-analysis, without any involvement of the manufacturer, that showed a small beneficial effect on blood pressure with unclear clinical relevancy of using device guided breathing^[8]. As was discussed by the authors of this meta-analysis, the overall effect estimate could have been biased due to inclusion of inadequately controlled trials and sponsored studies. In studies with

an acceptable methodological quality, no beneficial effects were seen. Sensitivity analysis showed that studies, performed without involvement of the manufacturer, showed no beneficial effects of device-guided breathing^[8]. Since the meta-analysis was published, 1 additional study has been completed. This study, which had a successful double-blinding procedure and a sham control group, showed no beneficial effects and even possible adverse events^[20]. Unfortunately, the writing group of the AHA guideline on non-pharmacological hypertension treatment finished writing the guideline before publication of this latest trial. As this latest study has the highest level of evidence, the writing group from the AHA was asked to reconsider their recommendation from Class II A, Level of Evidence B into class III, Level of Evidence B (evidence that treatment is not effective)^[25]. The committee responded that they didn't believe that the recommendation should be changed^[26]. Despite the fact that the latest study showed possible adverse events, the writing group focussed on a small positive general effect estimate from the meta-analysis by Mahtani *et al*^[8] and a meta-analysis that was performed by themselves^[4]. This positive recommendation by the guideline committee does not seem to be in line with the evaluation of the authors of the Mahtani *et al*^[8] study who criticized the methodological quality of most studies and the sponsor involvement in the discussion section of that paper^[8]. Since 1 member, who was involved in evaluating the topic of device-guided breathing for the AHA guideline, previously received funding from the manufacturer of the Resperate® device, the response of the AHA guideline committee is of potential concern^[4]. We agree with Mahtani *et al*^[8] that there is a real possibility that bias was introduced in the overall effect estimate from combining not adequately controlled studies and by including studies with a high level of sponsor involvement.

CONCLUSION

We conclude that, based on studies with acceptable methodological quality, there is no evidence for a short-term beneficial effect on blood pressure by using device-guided breathing. A meta-analysis of individual patient data combining studies with adequate control groups should be performed in the near future. Since there are no trials, not even uncontrolled, with sufficient follow-up on the feasibility and safety of using the device for many months or years, this device cannot safely be advised for treating hypertension in daily practice.

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WJC 6th Anniversary Special Issues (1): Hypertension

Hypertension and chronic ethanol consumption: What do we know after a century of study?

Katia Colombo Marchi, Jaqueline Joice Muniz, Carlos Renato Tirapelli

Katia Colombo Marchi, Programa de pós-graduação em Farmacologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, São Paulo, CEP 14040-900, Brazil

Jaqueline Joice Muniz, Carlos Renato Tirapelli, Departamento de Enfermagem Psiquiátrica e Ciências Humanas, Laboratório de Farmacologia, Escola de Enfermagem de Ribeirão Preto, Universidade de São Paulo, São Paulo, CEP 14040-902, Brazil

Author contributions: Marchi KC, Muniz JJ and Tirapelli CR solely contributed to this paper.

Correspondence to: Carlos Renato Tirapelli, PhD, Departamento de Enfermagem Psiquiátrica e Ciências Humanas, Laboratório de Farmacologia, Escola de Enfermagem de Ribeirão Preto, Universidade de São Paulo, Avenida Bandeirantes 3900, São Paulo, CEP 14040-902, Brazil. cartirapelli@eerp.usp.br

Telephone: +55-16-36020532 Fax: +55-16-3633-3271

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Abstract

The influences of life habits on the cardiovascular system may have important implications for public health, as cardiovascular diseases are among the leading causes of shorter life expectancy worldwide. A link between excessive ethyl alcohol (ethanol) consumption and arterial hypertension was first suggested early last century. Since then, this proposition has received considerable attention. Support for the concept of ethanol as a cause of hypertension derives from several epidemiologic studies demonstrating that in the general population, increased blood pressure is significantly correlated with ethanol consumption. Although the link between ethanol consumption and hypertension is well established, the mechanism through which ethanol increases blood pressure remains elusive. Possible mechanisms underlying ethanol-induced hypertension were proposed based on clinical and experimental observations. These mechanisms include an increase in sympathetic nervous system activity, stimulation of the

renin-angiotensin-aldosterone system, an increase of intracellular Ca^{2+} in vascular smooth muscle, increased oxidative stress and endothelial dysfunction. The present report reviews the relationship between ethanol intake and hypertension and highlights some mechanisms underlying this response. These issues are of interest for the public health, as ethanol consumption contributes to blood pressure elevation in the population.

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Key words: Ethanol; Hypertension; Calcium; Nitric oxide; Oxidative stress

Core tip: After a century of study, the relationship between chronic ethanol consumption and hypertension is well established. This review provides a description of the main studies that showed a relationship between chronic ethanol consumption and hypertension in humans. We also discuss studies using animal models of ethanol-induced hypertension, describing the main mechanisms by which ethanol consumption leads to hypertension.

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INTRODUCTION

Hypertension is a major independent risk factor for cardiovascular disease. In ethanol-consuming populations, the amount of ethanol consumed has a significant impact on blood pressure values, the prevalence of hypertension, and cardiovascular and all-cause mortality. The observa-

Table 1 List of the main epidemiological studies describing the relationship between ethanol consumption and hypertension

Ref.	Yr	Study	Subjects	Age (yr)
Lian ^[1]	1915		150	42-43
Clark <i>et al</i> ^[2]	1967	Los angeles heart	865	21 ¹
Gyntelberg <i>et al</i> ^[3]	1974	Copenhagen	5249	40-59
Klatsky <i>et al</i> ^[4]	1977	Kaiser-Permanente I	83947	15-79
Dyer <i>et al</i> ^[5]	1977	Chicago W. Electric	1899	40-55
Arkwright <i>et al</i> ^[6]	1982	Perth	491	20-45
Milon <i>et al</i> ^[7]	1982	Lyon	1134	20-59
Klatsky <i>et al</i> ^[10]	1986	Kaiser-Permanente II	66510	-

¹Mean age.

tion that the excessive consumption of ethyl alcohol (ethanol) is associated with high blood pressure is nearing its centennial mark^[1]. In the last century, numerous epidemiologic studies have found an association between ethanol consumption and arterial hypertension^[2-6]. It is estimated that 5% to 24% of hypertension cases are associated with ethanol consumption^[7,8]. However, although the link between ethanol consumption and arterial hypertension is well established, the mechanism through which ethanol increases blood pressure remains elusive. The effects of ethanol on the cardiovascular system are complex, and attempts to evaluate the possible mechanisms underlying ethanol-induced hypertension in humans are hindered by several limitations. These difficulties include differences in the duration of ethanol use, the timing and frequency of blood pressure measurements, variability in the type and frequency of ethanol intake, age, gender, ethnicity, salt use, body mass index and comorbid conditions.

Animal models of alcoholism may be relevant to understanding the mechanisms by which ethanol consumption increases blood pressure. Data support the involvement of increased sympathetic activity, stimulation of the renin-angiotensin-aldosterone system, increased intracellular Ca^{2+} in smooth muscle with a subsequent increase in vascular reactivity, oxidative stress and endothelial dysfunction. In this review, we will discuss the relationship between ethanol intake and hypertension and some of the possible mechanisms underlying this response. For the present review, a MEDLINE-based search was conducted using the following keywords: “alcohol”, “alcoholism”, “ethanol”, “blood pressure”, “hypertension”, “nitric oxide”, “oxidative stress”, “calcium”, “endothelial dysfunction” and “vascular reactivity”. Articles were further limited to those published in English (except the classic article published in French by Camille Lian) and containing abstracts. Reasons for the exclusion of articles include unclear ethanol dose or ingestion period. Information analysis started with the title, followed by the abstract and, finally, the complete report.

ETHANOL CONSUMPTION AND HYPERTENSION IN HUMANS (TABLE 1)

In 1915, the French army physician Camille Lian studied

approximately 150 French career soldiers (42 and 43 years old), relating their drinking to high blood pressure. The results of this study showed a clear threshold relationship of heavy drinking to hypertension, which was defined as 150/100 mmHg, and very heavy drinking increased the risk further. The moderate drinkers consumed 2 L of wine per day, the heavy drinkers consumed more than 2 L per day, and the very heavy drinkers consumed 3 or more liters per day. This was the first report on this relationship, but the result was ignored for approximately 50 years. In the 1960s and 1970s, findings among smaller patient populations corroborated the initial results described by Lian^[2,3].

In this review, for the purpose of standardization, the levels of ethanol consumption in humans have been expressed as the number of standard drinks per day (1 standard drink is defined here as the equivalent of 14 g of ethanol). A landmark observational study published in 1977, the Kaiser-Permanente Multiphasic Health Examination Data, reported differences in systolic blood pressure as high as 11 mmHg in individuals consuming 6 or more drinks per day compared with non-drinkers^[4]. This study was based on self-administered questionnaires from more than 80000 men and women and showed that a threshold of 3 or more drinks per day was a risk factor for hypertension across races and in both sexes. Moreover, the study found a relationship between the amount of ethanol consumed and blood pressure. This observation was corroborated by other studies. For example, among Danish men aged 40-59 years, the differences in blood pressure between those consuming 6 or more drinks per day and those consuming fewer drinks per day were 8 mmHg (systolic) and 4.5 mmHg (diastolic)^[5]. Systolic pressure increased progressively with increasing ethanol consumption among 491 Caucasian males aged 20-45 years. Importantly, the effect of ethanol on systolic blood pressure was independent of the effects of age, obesity, cigarette smoking and physical activity^[9].

The second Kaiser-Permanente study reconfirmed the relationship of higher blood pressure to ethanol use^[10]. Data from approximately 80000 persons, collected in the United States from 1978 to 1981, revealed a direct positive relationship between the regular consumption of alcoholic beverages and higher blood pressure, independent of potential confounding factors, including age, body weight and smoking status. One important finding of this study was that at 1 to 2 drinks per day, there was a slight but significant increase in blood pressure, indicating that the threshold was lower than that reported in the first Kaiser-Permanente study. The change in the threshold values between the two studies was the result of the division of lighter drinkers into several categories in the second study. As observed previously in the first Kaiser-Permanente study, systolic and diastolic blood pressures substantially increased at 3 to 5 and 6 or more drinks per day.

In his review of studies examining the prevalence of hypertension in ethanol consumption groups, MacMahon (1987) analyzed 29 cross-sectional studies and 6 prospec-

tive studies conducted in populations from a variety of geographic regions, including North America, Australia, Japan, Europe and New Zealand. Most of these studies reported a significant positive association between hypertension and ethanol consumption^[11]. The association was shown to be independent of confounders such as age, body mass index, smoking status and exercise. In general, the studies highlighted that the increase in systolic pressure was greater than that in diastolic pressure and that there was a trend toward a greater effect of ethanol on blood pressure in older men compared with younger men. Finally, the studies showed that at 3 to 4 drinks per day, the prevalence of hypertension was approximately 50% greater than that in non-drinkers, and at 6 to 7 drinks per day, the prevalence was 100% greater.

The exact threshold for the effect of ethanol on blood pressure is not clear. In fact, the threshold question is controversial, as epidemiologic studies could not resolve the question of a possible threshold for the apparent risk of hypertension. While several studies have suggested little or no effect of up to 1 or 2 drinks per day on blood pressure^[2-4,12], others have shown a progressive linear association^[6,7,13]. The first Kaiser-Permanente study described a threshold relationship at 3 to 5 drinks a day for men, with a substantial increase in systolic blood pressure at 6 drinks a day^[4]. However, the threshold was found to be at a much lower drinking level than that described in the first Kaiser-Permanente study. Significantly higher systolic pressures were found in Caucasian males who consumed 2 or fewer drinks a day^[9]. The second Kaiser-Permanente study described that at 1-2 drinks per day, there was a slight but significant increase in blood pressure^[4]. A slight increase in blood pressure was found in men reporting as few as 1 to 2 drinks per day in that survey.

The contribution of ethanol consumption to the prevalence of hypertension is dependent upon the population studied and varies widely in different populations. In developed countries such as the United States and England, it has been estimated that as much as 30% of hypertension may be attributed to ethanol consumption^[14]. Other studies suggested this proportion to be smaller. The Australian Risk Factor Prevalence Study^[15] estimated that 7% of the prevalence of hypertension could be attributed to ethanol consumption, while the first Kaiser Permanente Study estimated a proportion of 5%^[4]. In these two studies, it was estimated that a maximum of 11% of hypertension in men could be attributed to the consumption of ethanol. A French epidemiological study estimated that 24% of the prevalence of hypertension in French men could be attributed to ethanol consumption^[7]. Similar results were found in a cross-sectional study in Sidney, where it was estimated that 24% of hypertension may be attributed to ethanol consumption^[16].

The estimate is somewhat lower in women and higher in men^[4,10]. In the Risk Factor Prevalence Study^[15], ethanol consumption accounted for no more than 1% of hypertension in women. The reasons for the gender

difference in the proportion of hypertension prevalence associated with ethanol consumption are not fully understood, but they are most likely attributed to the less consumption of ethanol by women than men^[11].

The mechanism(s) by which ethanol consumption leads to elevations in blood pressure is uncertain. A small number of studies in humans have attempted to address this question. The role of catecholamines in mediating the effects of ethanol on blood pressure has been investigated in humans. In this regard, increases in plasma adrenaline^[17] and noradrenaline^[18] were described in humans after ethanol ingestion, and it was suggested that activation of the adrenergic system may be responsible for the increased blood pressure. On the other hand, Potter *et al.*^[19] did not observe changes in catecholamines levels after ethanol consumption. Moreover, these authors reported that plasma renin and cortisol levels were not affected by the consumption of ethanol^[19]. Arkwright *et al.*^[9] observed that, although blood pressure was higher among ethanol drinkers, there were no changes in plasma adrenaline, noradrenaline, cortisol and renin in these subjects. Conversely, Ibsen *et al.*^[20] showed increased plasma renin levels among heavy ethanol drinkers. Potter *et al.*^[19] found that plasma cortisol, but not plasma rennin, increased after ethanol consumption. The reason for the inconsistencies among these results is uncertain, and further studies on the mechanisms underlying the pressor effects of ethanol in humans would be of value. The results of these studies raise a number of possibilities concerning the involvement of humoral mechanisms in the pressor effects of ethanol. However, the available data in humans are not sufficient to allow substantive conclusions. In light of the need for careful investigation of the mechanisms underlying the effects of ethanol on blood pressure, experimental models were created and are used for this purpose.

ANIMAL MODELS OF ETHANOL-INDUCED HYPERTENSION

Most experimental studies corroborate the findings of the epidemiological studies described above, confirming that ethanol consumption is associated with increased blood pressure levels and an increased prevalence of hypertension. Chan and Sutter^[21] found that treatment of male Wistar rats for 12 wk with a solution of ethanol (20% *v/v*) resulted in mild hypertension. An increase of approximately 25% in mean arterial blood pressure (from 98 to 122 mmHg) was described later by these authors using the same experimental model^[22]. Similarly, Abdel-Rahman *et al.*^[23] observed an increase in systolic blood pressure after 12 wk of ethanol feeding (20% *v/v*) in Wistar and Sprague-Dawley rats. Blood pressure was significantly higher at week 6 in Sprague-Dawley ethanol-fed rats (from 106 to 147 mmHg) and at week 8 in Wistar ethanol-fed rats (from 117 to 149 mmHg). The authors also found that ethanol-fed rats had a higher sympathetic activity, as beta-blockade with propranolol decreased heart rate to

a greater degree in ethanol-fed rats than it did in control rats^[23]. Strickland and Wooles^[24] showed that the systolic and diastolic pressures of ethanol-fed (ethanol 20% *v/v*) Sprague-Dawley rats became significantly greater at 4 wk and continued to increase throughout the remainder the study. The systolic blood pressure of ethanol-fed rats was increased by 6.6 mmHg at 4 wk and by 33.8 mmHg at 22 wk compared with the controls. The difference in diastolic blood pressure between the control and ethanol-fed rats was 5.8 mmHg at 4 wk, and this difference increased to 47 mmHg by 22 wk of ethanol feeding^[24]. Vasdev *et al*^[25-27] described an increase in systolic blood pressure in male Wistar rats after 1 wk of treatment with ethanol. The rats were given 5% ethanol in their drinking water for 7 wk, and the systolic blood pressure in the ethanol-treated rats was found to be significantly higher than that in the controls after 1 wk or longer^[25-27]. Interestingly, the discontinuation of ethanol treatment for 7 wk did not reverse the hypertension or the adverse renal vascular changes in ethanol-induced hypertensive rats^[25].

In the study of Utkan *et al*^[28], systolic blood pressure was recorded weekly using the tail-cuff method in Wistar rats treated with ethanol (7.2% *v/v*) for 4 wk. There was a mild but significant elevation of systolic blood pressure in the ethanol-fed rats by week 1 compared to baseline measurements, and this difference remained higher at later times. This study showed that the hypertensive state associated with ethanol intake can be observed in the early stages of ethanol consumption. A possible explanation for such a finding could be the higher blood ethanol levels found in this study (293.6 ± 5.2 mg/dL)^[28]. Brown *et al*^[29] showed that ethanol-consuming Sprague-Dawley rats exhibited elevated systolic blood pressures compared with the control group (151.6 ± 0.6 *vs* 132.9 ± 2.7 mmHg). In this study, the blood ethanol levels averaged 63.8 ± 2.5 mg/dL.

In a previous study, we compared the effects of ethanol intake (20% *v/v*) for 2, 6 and 10 wk on arterial blood pressure in conscious Wistar rats^[30]. The baseline systolic, diastolic and mean arterial pressure values of ethanol-treated rats were increased (approximately 20%) after the 3 different periods of treatment. Because blood pressure was already elevated in the 2-wk-treated rats, our results supported the notion that the hypertensive state associated with ethanol intake can occur in the early stages of ethanol consumption. This finding contrasted those of previous studies, which have reported that blood pressure elevation occurred late during chronic ethanol treatment^[23,24,28]. Blood ethanol content is a potential explanation for the disparity among reports.

Using this same model of ethanol feeding, we investigated the effects of ethanol treatment for 2 and 6 wk on both blood pressure and vessel reactivity. Mild hypertension was observed in chronically ethanol-treated rats, which was due to increases in both systolic and diastolic pressures. Chronic ethanol consumption in rats increased the contractile response of the aorta and mesenteric arterial bed^[31-33]. In addition to its hypertensive effect, ethanol consumption can also modulate the response to vaso-

active agents *in vivo*. Data from our group showed that chronic ethanol consumption increased blood pressure as well as the pressor response induced by phenylephrine and endothelin-1^[30,34].

The studies using animal models established a positive correlation between the duration of ethanol consumption and the increase in blood pressure, showing that the period of exposure to ethanol is an important factor in the development of hypertension^[23,24]. Additionally, there is evidence that blood ethanol concentration contributes to the increase in blood pressure in animal models of alcoholism, where higher blood ethanol concentrations may account for the earlier development of hypertension. Previously, we showed that increased blood pressure, concomitant with ethanol feeding, was observed in 2-wk ethanol-treated animals, in which the blood ethanol content was 1.67 ± 0.21 mg/mL^[30]. Abdel-Rahman *et al*^[23] reported a blood ethanol concentration of 0.53 ± 0.04 mg/mL in 12-wk-treated rats. Additionally, Abdel-Rahman *et al*^[23] (1985), who did not detect blood pressure changes after ethanol treatment, reported a blood ethanol concentration of 0.34 ± 0.04 mg/mL in rats treated with ethanol for 30 d^[35].

Several mechanisms have been postulated for the hypertensive response to chronic ethanol consumption. Evidence suggests the existence of a myogenic mechanism(s) that involves alterations in the contractile/relaxant properties of vascular smooth muscle. In fact, the majority of studies describing the effects of ethanol on arterial blood pressure also evaluated the effects of ethanol on vascular responsiveness^[24,28,29,31-33].

MECHANISMS UNDERLYING ETHANOL-INDUCED HYPERTENSION (TABLE 2)

Myogenic mechanism

Much of the research investigating the chronic effects of ethanol on the cardiovascular system has addressed vascular responsiveness to vasoconstrictor agents. In this regard, enhanced vascular reactivity to vasoconstrictor agents or impairment of vascular relaxation is described to contribute to the cardiovascular complications associated with chronic ethanol consumption. The initial studies in this field showed enhanced vascular reactivity to α_1 -adrenoceptor agonists in different arteries from ethanol-fed rats. Pinardi *et al*^[36] found that chronic ethanol consumption significantly enhanced the contractile response induced by phenylephrine of endothelium-intact aortic rings. Noradrenaline-induced contraction of the superior mesenteric artery was shown to be greater in rings from ethanol-treated rats^[37]. Likewise, there was an ethanol-associated increase in the maximal contractile response to phenylephrine, a selective α_1 -adrenoceptor agonist, in endothelium-denuded aortic rings^[38]. Later, Ladipo *et al*^[39] demonstrated that chronic ethanol consumption increased the sensitivity of rat aortic rings to noradrenaline. At this point, although it was well estab-

Table 2 Summary of the main mechanisms underlying ethanol-induced hypertension

Ref.	Mechanism
[17,18] [20]	Increase in sympathetic nervous system activity Stimulation of the renin-angiotensin-aldosterone system
[31,32,36-42]	Myogenic mechanism: Enhanced vascular reactivity to vasoconstrictor agents
[33,41,44-46]	Impairment of the vascular relaxation Oxidative stress:
[70-77]	Increase in reactive oxygen species generation
[81,82,85-87]	Reduction of antioxidant systems
[28,44,52,95-102]	Decrease of nitric oxide bioavailability and endothelial dysfunction

lished that chronic ethanol consumption enhanced α_1 -induced contraction, the mechanisms underlying this response were poorly understood. Moreover, the experiments designed to study the vascular effects of chronic ethanol consumption on α_1 -induced contraction used only one period of treatment^[21,28,29]. Based on these observations, we proposed a study to investigate the time-course of changes in vascular reactivity to phenylephrine in aortas from chronically ethanol-treated rats as well as to evaluate in detail the mechanisms underlying the effects of long-term ethanol consumption on α_1 -induced contraction. Chronic ethanol consumption produced an increased responsiveness to phenylephrine in aortas, although there was no relationship between the period of treatment (2, 6 and 10 wk) and the magnitude of the enhancement of α_1 -induced contraction^[40]. Importantly, the increased responsiveness to phenylephrine was also observed after endothelial denudation, further suggesting that the increased sensitivity to α_1 -adrenergic agonists was not dependent on the presence of the endothelium. The enhanced vascular response to phenylephrine observed in the aorta of ethanol-treated rats was maintained by two mechanisms: an increased release of thromboxane A₂, a vascular smooth muscle-derived vasoconstrictor prostanoid, and an increased extracellular Ca²⁺ influx. One interesting finding of this study was that the increased response to phenylephrine was not the result of a nonspecific increase in rat aorta reactivity induced by chronic ethanol intake, as the contractile responses to endothelin-1 or KCl were not affected by the ethanol treatment. In fact, while studying the effect of ethanol consumption on the reactivity of rat carotids to endothelin-1, we found an increase in endothelin-1-induced contraction in this artery with no change in the contraction induced by phenylephrine^[41,42]. The hyperactivity to endothelin-1 in the rat carotid was not different among the three periods of treatment (2, 6 and 10 wk) used in our study. The potentiation of endothelin-1-induced contraction in the rat carotid was caused by reduced expression of pro-relaxation endothelial endothelin receptor type B (ET_B) receptors.

Most of the experiments designed to study the relationship between alterations in vascular functionality and increases in blood pressure induced by ethanol consump-

tion used conduit vessels, such as the aorta. However, while the aorta does not offer substantial resistance to blood flow, the contribution made by vessels of smaller diameter to peripheral vascular resistance is much greater. In rats, the mesenteric circulation receives approximately one-fifth of the cardiac output^[43], and thus, regulation of this bed provides a significant contribution to the regulation of systemic blood pressure. To further analyze this aspect, we evaluated whether alterations in the reactivity of the mesenteric arterial bed could account for the hypertensive state associated with ethanol consumption^[31]. Chronic ethanol consumption produced an endothelium-dependent increased responsiveness to phenylephrine in a perfused mesenteric arterial bed isolated from rats treated with ethanol for 6 wk but not from rats treated for 2 wk. However, increased blood pressure was observed in ethanol-treated animals after 2 wk, whereas altered responsiveness to phenylephrine was only observed in rats treated for 6 wk. These observations supported the notion that the altered responsiveness of resistance arteries was not the cause, but rather the consequence, of the increased blood pressure associated with ethanol intake^[31,32]. The increased vascular response to phenylephrine observed in the mesenteric arterial bed was maintained by two mechanisms: an increased release of endothelial-derived vasoconstrictor prostanoids and a reduced modulatory action of endothelial nitric oxide (NO); the latter is likely associated with a reduced expression of the enzyme eNOS (endothelial NO synthase)^[32].

Impairment of vascular relaxation may also contribute to the cardiovascular complications associated with chronic ethanol consumption. Long-term ethanol consumption significantly reduced acetylcholine-induced relaxation in the aortic rings from rats treated with ethanol for 12 wk^[44] and 8 wk^[45]. In the rat carotid, the relaxation induced by IRL1620, a selective endothelin ET_B receptor agonist, was reduced after treatment with ethanol; this effect was mediated by a mechanism involving the down-regulation of endothelial ET_B receptors^[41]. More recently, we found that chronic ethanol consumption reduced the endothelium-dependent relaxation induced by the peptide adrenomedullin in the rat aorta^[46].

In resistance arteries, Hatton *et al.*^[37] showed an increased response of mesenteric arteries to noradrenaline in rats treated with ethanol for 18 wk. The finding that the relaxation induced by acetylcholine, but not by sodium nitroprusside, was reduced in the mesenteric arterial bed from ethanol-treated rats indicated that chronic ethanol consumption decreased the action of NO or its endothelial cell receptor-stimulated production/release^[32]. Similarly, ethanol consumption was also found to reduce the endothelium-dependent relaxation induced by adrenomedullin in the rat mesenteric arterial bed^[33]. The vascular relaxation induced by adrenomedullin in the rat mesenteric arterial bed is endothelium-dependent and involves the activation of the NO-cyclic guanosine monophosphate pathway^[47]. In our study, no differences in adrenomedullin-induced relaxation were detected in control and ethanol-exposed tissues after incubation with

L-nitro-arginine methyl ester, a NOS inhibitor, suggesting that the reduced adrenomedullin responsiveness of the mesenteric arterial bed from ethanol-treated rats was due to an impaired modulation of adrenomedullin-induced relaxation by NO^[33].

The vascular endothelium and vascular smooth muscle cells are important targets for the effects of ethanol consumption. These effects are complex, and the identification of biochemical/molecular mechanisms that could explain such effects is warranted. A number of mechanisms have been postulated to explain the pathogenesis of high-dose ethanol toxicity in the vasculature. These mechanisms include an increase in intracellular Ca²⁺ levels with a subsequent increase in vascular reactivity, oxidative stress and a reduction in NO bioavailability. These processes will be discussed in the following sections.

Alterations in Ca²⁺ levels

One of the mechanisms by which chronic ethanol consumption leads to alterations in vascular responsiveness is by increasing the intracellular Ca²⁺ levels in vascular smooth muscle cells. Ca²⁺ is a cation of critical importance for many cellular control mechanisms, including muscle contraction. During excitation, the intracellular Ca²⁺ concentration increase by either (1) Ca²⁺ entry through the plasma membrane through voltage- or ligand-gated ion channels, or (2) release from intracellular stores (sarcoplasmic reticulum or mitochondria).

Some studies have provided evidence that ethanol consumption increases the intracellular Ca²⁺ concentration. This response may result from a direct effect of ethanol on plasma membrane permeability, Na⁺ transport and Na⁺-Ca²⁺ exchange, and/or impaired Ca²⁺ transport due to a secondary abnormality, such as Mg²⁺ depletion, which is described in alcoholics^[48]. Increased Ca²⁺ influx results in increased vascular contractility and reactivity, and those responses increase vascular tone and peripheral vascular resistance, thereby elevating blood pressure^[49]. Tirapelli *et al.*^[40] described an increased phenylephrine-induced contractility of arteries from ethanol-treated rats. SQ29548, a potent and selective thromboxane A2 receptor antagonist, reduced the maximal CaCl₂ response of aortic rings from ethanol-treated rats, suggesting that the enhanced response to extracellular Ca²⁺ was modulated by PGH₂/TXA₂. Based on these results, it was concluded that prostanoids mediate the enhanced reactivity to phenylephrine by mechanisms that alter the mobilization of or sensitivity to extracellular Ca²⁺^[40].

The effect of chronic ethanol administration on blood pressure and its relation to Ca²⁺ were also investigated by Hsieh *et al.*^[50] in 7-wk-old Wistar rats that had received 15% ethanol in their drinking water. The blood pressure in ethanol-treated rats was significantly higher than in the controls. The extracellular fluid volume was increased in ethanol-treated rats, and the blood pressure significantly correlated with increases in the intracellular Ca²⁺ concentration. These results suggest that increased intracellular Ca²⁺ and augmented body fluid volume contributed to the development of ethanol-induced hyper-

tension. It was also suggested that these responses were partly mediated by Mg²⁺ depletion and suppressed Na⁺ pump activity^[50]. In fact, these factors appear to be all-important in the etiology of hypertension^[51].

In 2008, Tirapelli *et al.*^[52] reported an increased responsiveness to KCl of arteries from female rats chronically treated with ethanol. Because KCl-induced contraction depends almost exclusively on Ca²⁺ influx through the activation of voltage-sensitive channels^[53], it was suggested that ethanol consumption increases the Ca²⁺ influx through these channels. Vasdev *et al.*^[54] observed that ethanol consumption (10% ethanol in drinking water-6 wk) increased systolic blood pressure and that this response was associated with an increased Ca²⁺ uptake by aortas from ethanol-treated animals. These findings suggested that increases in cytosolic free Ca²⁺ and in Ca²⁺ uptake in the vasculature are associated with ethanol-induced hypertension. Two years later, these authors reported that verapamil, a Ca²⁺ channel blocker, reversed the increase in systolic blood pressure and aortic Ca²⁺ uptake induced by chronic ethanol consumption. In addition to the effects observed previously, the authors observed smooth muscle cell hyperplasia in small arteries and in renal arterioles from ethanol-treated rats^[25].

In a clinical study, it was demonstrated that both systolic and diastolic blood pressures were significantly higher in individuals drinking 275 g ethanol per week^[55]. In these subjects, increased plasma Ca²⁺ levels were correlated with increased diastolic blood pressure. An increment in diastolic pressure of 6.9 mmHg correlated with increments of 0.1 mmol/L in plasma Ca²⁺ concentration. Those findings suggested that regular ethanol consumption predisposes to hypertension by facilitating Ca²⁺ accumulation in cells involved in blood pressure regulation^[55]. Taken together, the above-mentioned studies suggest a role for Ca²⁺ in ethanol-induced hypertension. In this scenario, ethanol consumption would alter Ca²⁺ influx/permeability in the vasculature with a consequent increase in vascular contractility and peripheral resistance, which in turn would be responsible for the increase in blood pressure associated with ethanol consumption.

Oxidative stress

Reactive oxygen species (ROS) are reactive chemical entities produced as intermediates in reduction-oxidation (redox) reactions. Perturbations of the balance between ROS production and scavenging by antioxidant systems result in oxidative stress and presumably in pathophysiological changes. Oxidative stress is a common mediator of pathogenicity in cardiovascular diseases, such as hypertension^[56,57]. ROS have an important pathophysiological role in inflammation (by influencing platelet aggregation and migration of monocytes), hypertrophy, proliferation, fibrosis, angiogenesis, processes that are involved in cardiovascular remodeling and endothelial dysfunction^[58-61].

The role of ROS in the pathophysiology of hypertension is well established^[62-64]. The causal relationship between ethanol, ROS and hypertension most likely occurs at the vascular level, where ethanol promotes oxidative

stress, endothelial dysfunction, vascular inflammation, increased vascular reactivity and structural remodeling. Together, these responses lead to increased peripheral resistance and therefore to increased blood pressure^[65, 66]. It is known that ROS modulate specific cellular pathways (redox signaling), leading to changes in gene transcription and in functional oxidative modifications of cellular proteins that cause cellular dysfunction^[56, 67, 68]. Thus, oxidative stress not only causes direct and irreversible oxidative damage to macromolecules, but it also affects redox-dependent signaling in the vasculature^[69]. ROS generation by ethanol is important to its pathophysiology in the cardiovascular system, as ethanol is extensively metabolized into acetaldehyde in the liver, mainly by the enzyme alcohol dehydrogenase^[70]. Acetaldehyde, in turn, is oxidized to acetate by acetaldehyde dehydrogenase, which results in the generation of ROS and decreased NO levels^[71].

In addition to the ROS generated during ethanol metabolism, some studies have shown the involvement and contribution of the nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidases to dysfunctions promoted by chronic ethanol consumption in several tissues^[72-76]. Increased vascular oxidative stress induced by ethanol consumption is related to the activation of the enzyme NAD(P)H oxidase, and this mechanism is involved in the increased blood pressure caused by chronic ethanol consumption. NAD(P)H oxidase is the main source of ROS in endothelial and smooth muscle vascular cells^[65], and it is considered a key factor in the vascular dysfunctions induced by ethanol. Husain *et al.*^[77] demonstrated that chronic ethanol consumption leads to an increased NAD(P)H oxidase activity and ROS generation that leads to membrane lipid peroxidation. The authors also observed increased phenylephrine-induced contraction and reduced acetylcholine-induced relaxation in aortas from ethanol-treated rats^[77]. These data suggest that the initial step in the cardiovascular dysfunction associated with chronic ethanol consumption involves the formation of ROS, and this process can be mediated by the enzyme NAD(P)H oxidase. Moreover, this enzyme has been implicated in the activation of xanthine oxidase and the uncoupling of eNOS, which leads to ROS overproduction^[78].

The antioxidant enzymes are the first line of defense against ROS-induced oxidative tissue injury. In vascular tissue, the enzymatic antioxidant system mainly consists of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), thioredoxins and peroxiredoxins. The non-enzymatic antioxidants include ascorbate, tocopherol, glutathione, bilirubin and uric acid^[79, 80]. The antioxidant mechanisms antagonizing the consequences of chronic ethanol consumption have particularities related mainly to the type of tissue studied, the duration of treatment and the concentration of ethanol used. Das and Vasudevan^[81] showed that ethanol consumption increased SOD activity and decreased CAT activity in a time- and dose-dependent manner^[81]. Husain *et al.*^[82] demonstrated increased SOD activity in the liver of rats treated with ethanol^[82]. It is known that SOD activity is modulated by

increased ROS generation and by lipid peroxidation^[83, 84]. In rats, chronic ethanol treatment led to increased CAT activity and impaired the maintenance of the glutathione redox cycle in renal tissue, with an increase in GPx activity and a decrease in GSH (reduced glutathione) levels^[84].

In clinical studies, increased plasma activity of SOD and GPx was observed in subjects who regularly consume ethanol^[85, 86]. Husain *et al.*^[87] demonstrated that chronic ethanol consumption by rats significantly depressed both cytosolic CuZn-SOD and mitochondrial Mn-SOD activities in the plasma, indicating an inability of the cells to scavenge superoxide anion. Moreover, plasma CAT and GPx activities were also significantly decreased in ethanol-treated rats. The inhibition of these enzymes may increase superoxide anion availability, which can react with NO to form peroxynitrite^[87].

The role of oxidative stress in ethanol-induced hypertension is complex and may involve increases in ROS generation or reductions in antioxidant systems. The increase in oxidative stress promoted by ethanol is associated with endothelial dysfunction, vascular inflammation and increased vascular reactivity. These processes may contribute directly or indirectly to increased peripheral resistance and therefore to increased blood pressure.

NO bioavailability

In 1980, Furchgott *et al.*^[88], in classic study, discovered that endothelial cells produce an endothelium-derived relaxing factor (EDRF) in response to stimulation by acetylcholine. In 1987, Palmer *et al.*^[89] and Ignarro *et al.*^[90] identified EDRF as NO, a free radical that diffuses to underlying smooth muscle to induce vasodilatation^[89, 90]. These findings marked the beginning of a major worldwide expansion of research into the role of NO in vascular physiology and pathophysiology.

The endothelium plays a pivotal role as a sensor, transducer, and integrator of signaling processes regulating vascular homeostasis, and it is known that vascular diseases, including hypertension, are characterized by impaired endothelium-derived NO bioactivity. The effect of ethanol on the function of the endothelium is complex^[91]. Appreciating the importance of NO in the maintenance of vascular tone, some studies have examined the mechanisms underlying the impairment of NO-mediated vasodilatation by chronic ethanol consumption^[92]. In theory, such a decrease in NO bioactivity could result from reduced NO production or from the inactivation of NO^[93]. NO is produced by NOS (nitric oxide synthase) *via* one of three isoforms: the neuronal NOS (nNOS/NOS1), inducible NOS (iNOS/NOS2), and the endothelial NOS (eNOS/NOS3)^[94]. Ethanol exerts different effects on these isoforms in a variety of cells and tissues. Tirapelli *et al.*^[52] demonstrated that chronic ethanol consumption reduced the vascular expression of eNOS in female rats. Conversely, iNOS expression in arteries from ethanol-treated rats was significantly increased compared with control tissues. This response could be the result of a compensatory mechanism, where increased iNOS expression could induce a substantial and sustained release of NO

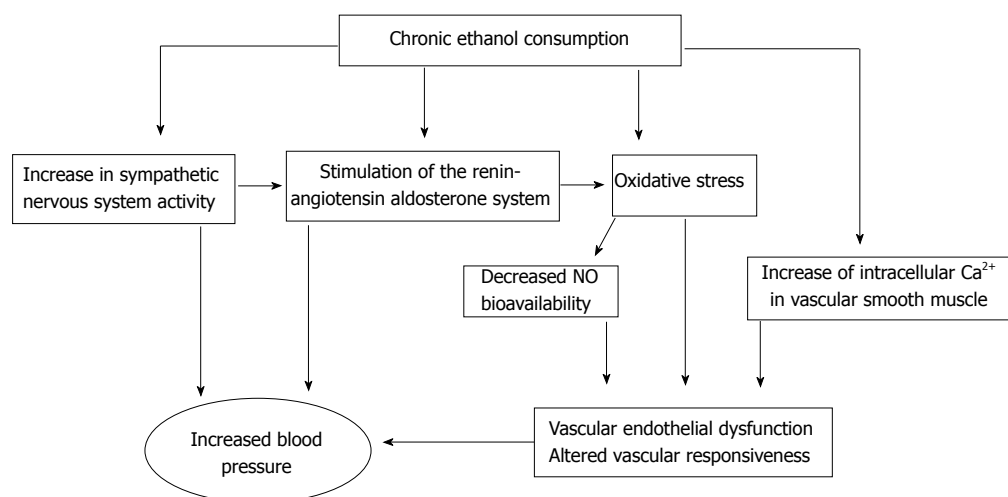


Figure 1 Summary of the basic pathophysiological mechanisms underlying ethanol-induced hypertension.

to compensate for the reduction of eNOS expression^[52]. In the rat liver, ethanol decreased eNOS expression and activity^[95]. Krecsmarik *et al.*^[96] demonstrated that chronic ethanol consumption induced an increase in iNOS activity and a decrease in nNOS expression in the rat gastrointestinal tract^[96]. Moreover, chronic ethanol treatment reduced the eNOS-dependent relaxation of cerebral arterioles in rats^[97].

The effect of ethanol on endothelial NO bioavailability appears to be related to the dose of ethanol. In this sense, it was shown that low concentrations of ethanol induced an increased release of endothelial NO due to the activation and expression of NOS^[98,99]. Utkan *et al.*^[28] described that chronic ethanol consumption potentiates endothelium-dependent relaxation in aortic rings, most likely through interference with the synthesis and/or release of NO or adaptive alterations in muscarinic receptors on the endothelial cells^[28].

While low concentrations of ethanol are described to increase endothelial NO production, the chronic consumption of high doses of ethanol impairs endothelial function in association with reduced NO bioavailability. Husain *et al.*^[44,100] described down-regulation of the NO-generating system, leading to impaired vasorelaxation and hypertension. Male Fisher rats orally administered 20% ethanol (4 g/kg - 12 wk) showed increased systolic and diastolic blood pressures and impaired vascular relaxation compared with controls. The expression of eNOS in the thoracic aorta isolated from ethanol-fed rats was down-regulated, leading to a depletion of aortic NO. This process may alter resistance vessel architecture, reducing its dilatatory capacity^[44,100]. In 2004, Kuhlmann *et al.*^[101] reported that high concentrations of ethanol decreased NO synthesis in and proliferation of endothelial cells from human umbilical veins.

The concentration of plasma asymmetric dimethylarginine (ADMA) in alcoholics is higher than in non-alcoholic subjects^[102]. ADMA is an endogenous inhibitor of NO production, which is generated from the methylation of arginine residues by arginine methyltransferases and

subsequent proteolysis. In this sense, increased ADMA levels could also contribute to the reduced bioavailability of NO in alcoholics.

NO, which is constantly formed, readily reacts with reactive molecules, such as superoxide anion^[103,104]. Most of the cytotoxicity attributed to NO is due to peroxynitrite, which is produced from the reaction between NO and superoxide anion^[105]. This loss of NO that occurs in the reaction with superoxide anion deprives vascular smooth muscle cells of NO. Ethanol reduces the bioavailability of NO through both the inhibition of eNOS and through the formation of peroxynitrite, which can lead to cellular damage^[106].

CONCLUSION

The link between hypertension and chronic ethanol consumption is well established, and the mechanism by which ethanol increases blood pressure is complex. There appears to be more evidence implicating the sympathetic nervous system, the renin-angiotensin-aldosterone system, increased intracellular Ca²⁺ in vascular smooth muscle, oxidative stress, decreased NO bioavailability and endothelial dysfunction than there is evidence for the other mechanisms suggested, but this issue remains an open one. After a century of study, it is established that chronic ethanol consumption leads to hypertension and that this process is a multi-mediated event involving the aforementioned mechanisms (Figure 1). Thus, it is of great importance to invest in implementing strategies that help to prevent alcoholism, thus reducing the risk of ethanol-associated cardiovascular diseases.

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WJC 6th Anniversary Special Issues (1): Hypertension

Asserted and neglected issues linking evidence-based and Chinese medicines for cardiac rehabilitation

Arthur de Sá Ferreira, Nathalia Gomes Ribeiro de Moura

Arthur de Sá Ferreira, Nathalia Gomes Ribeiro de Moura, Laboratory of Computation Simulation and Modeling in Rehabilitation, Postgraduate Program of Rehabilitation Science, Centro Universitário Augusto Motta/UNISUAM, CEP 21041-010, Rio de Janeiro, Brazil

Author contributions: Ferreira AS conceived the manuscript; Ferreira AS and Moura NGR revised the literature, drafted and approved the final version of the manuscript.

Correspondence to: Arthur de Sá Ferreira, PT, PhD, Laboratory of Computation Simulation and Modeling in Rehabilitation, Postgraduate Program of Rehabilitation Science, Centro Universitário Augusto Motta/UNISUAM, Praça das Nações 34, 3º andar, Bonsucesso, CEP 21041-010, Rio de Janeiro, Brazil. arthur_sf@ig.com.br

Telephone: +55-21-38829797 Fax: +55-21-25642244

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Abstract

High blood pressure is among the most prevalent chronic disease in adults that impacts on the quality of life of patients, which are often subjected to physical rehabilitation. Chinese medicine intervention in patients with hypertension presents promising albeit inconclusive results, mostly due to methodological issues. This paper discusses asserted and neglected issues linking evidence-based and Chinese medicines as related to systemic arterial hypertension, as well as their impact on the physical rehabilitation of those patients. On the one hand, natural history of hypertension, pulse palpation, and herbal therapy are among the asserted issues because of the scientific evidence collected about them, either in favor or against its integration to the current medical practice. On the other hand, anatomical variations of vessels and comparative physiology are among the most commonly neglected issues because previous researches on integrative medicine ignored the possible effects of these issues as related to the study's outcome. The asserted issues highlighted in this paper

stimulate the increasing use of Chinese medicine for health care and the continuity of research on integrative medicine in the cardiovascular field for rehabilitation. The neglected issues poses additional challenges that must not be overlooked in future research on this topic so that the integration of both traditional and current knowledge may be of benefit to the population with cardiovascular disease.

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Key words: Cardiovascular disease; Hypertension; Chinese medicine; Rehabilitation; Integrative medicine

Core tip: Integrative medicine might provide better clinical results than evidence-based or Chinese medicines isolated for patients undergoing cardiac rehabilitation. The asserted issues highlighted in this paper (natural history of hypertension, pulse palpation, and herbal therapy) stimulate the increasing use of Chinese medicine for health care and the continuity of research on integrative medicine in the cardiovascular field. Conversely, some neglected issues (anatomical variations of vessels and comparative physiology) poses challenges that must not be overlooked in future research on this topic so that the integration of both traditional and current knowledge may be of benefit to the population with cardiovascular diseases.

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INTRODUCTION

High blood pressure is a major public health problem

worldwide. Hypertension is among the most prevalent chronic, non-contagious disease in adults^[1], despite the trend to decrease its prevalence in some countries^[2]. The natural history of this disease still needs elucidation: although most of its modifiable and non-modifiable risk factors are well known, the etiology of primary systemic arterial hypertension (SAH) remains uncertain^[3]. The long-term impact of hypertension on health is nevertheless evident. Small, middle and large-sized arteries are the earliest body structures affected by time-sustained levels of high blood pressure^[4]. Such arterial remodeling process contributes to the pathophysiology of this condition in target-organs others than the arteries such as the skeletal muscle^[5], heart, kidneys, brain, and eyes^[6]. Without early and proper intervention, organic functions start to deteriorate such that they are detectable by either laboratorial or imaging exams as a complement to the clinical examination of signs and symptoms^[3]. On a timely fashion, functional capacity may be compromised at the systemic level^[7] with possible impacts on the quality of life of these patients^[8], which often are subjected to physiotherapy and cardiac rehabilitation.

Chinese medicine comprises a phenomenological, philosophic, and systematic traditional health care system developed through almost five millennia^[9]. Because Chinese medicine was rooted in a sociocultural environment that differed from the European medicine at its early beginning, it is reasonable to expect differences on both medical practices and respective evolution of medical theories. Nevertheless, recent randomized clinical trials, systematic reviews, and meta-analyses on the efficacy of Chinese medicine interventions in patients with SAH were conducted^[10] with promising albeit inconclusive results. In general, those studies help answering questions raised from the clinical point-of-view, such as “Is Chinese medicine intervention effective for reducing or controlling blood pressure levels”. Investigating this point-of-view leaves opened the traditional point-of-view, which raised questions such as “Are there actual subtypes of hypertension as related to Chinese medicine” or “Is the theory of pattern differentiation for diagnosis relevant for guidance on therapeutic intervention”.

In other words, one may argue what are the scientific evidences for the statements found in the Chinese medicine literature, specially the most antique ones. On the one hand, diving into the traditional Chinese medical literature one can find a number of traditional assertions calling for scientific evidence, if any. On the other hand, researchers often assume that some of these traditional factors may not have a detectable effect on their study's outcome. As it was argued that integrative medicine might provide better clinical results than either one isolated^[11], a comprehensive overview of the asserted and neglected issues between evidence-based and Chinese medicines is necessary for both clinicians and researchers. Therefore, this paper discusses the asserted and neglected issues linking evidence-based and Chinese medicines as related to SAH, as well as their possible impact on the physical rehabilitation of those patients.

ASSERTED ISSUES

In this section, the natural history of SAH, pulse palpation, and herbal therapy are discussed. These topics are considered as asserted issues because of the scientific evidence collected either in favor or against their integration into the current medical practice. However, they should not be considered as final positions because there are lacunas that still need to be addressed in future studies. Table 1 presents summary information about the studies cited in this section.

Natural history of SAH

The epidemiological concept of natural history of diseases also applies to Chinese medicine, with proper correspondence due to their inherent conceptual differences. The Chinese medicine counterpart of an ongoing morbid process is called *zheng* or pattern. It is worth noticing that a pattern encompasses other information than just signs and symptoms in the Western sense: behavior, emotional states, self-awareness of social status, and physical constitution are among other manifestations considered for diagnosis or “pattern differentiation”^[9]. Regardless of these differences, Chinese medicine theory presents basic elements of the natural history of diseases such as the existence of protection and risk factors for patterns, a clinical horizon for the onset of manifestations, and health outcomes such as cure, permanent or temporarily disability, and death.

As a matter of fact, there is evidence supporting that most clinical manifestations observed in patients with SAH and that are used for pattern differentiation are actually associated with target-organs damage (TOD). For instance, the clinical manifestations of cerebrovascular disease are strongly associated (Pearson correlation coefficient = 0.718, $P < 0.001$) to those of “Obstruction of phlegm and dampness of Heart/Liver/Gallbladder”^[12]. Moreover, long-term SAH can lead to myocardial ischemia, conduction defects, arrhythmias, and ventricular hypertrophy^[13]. The brain is another target-organ usually damaged by the SAH; cognitive disturbances in the elderly are, at least in part, hypertension-related^[14-16]. High risk of stroke, cognitive decline, and dementia are also associated to SAH^[17-19]. Some mild retinal changes are largely non-specific except in young patients, hemorrhages, exudates and papilledema, are only present in severe hypertension and are associated with increased cardiovascular risk^[3]. All the above-cited TOD eventually manifests signs and symptoms, which should be early detected in the natural history of SAH. Therefore, it is possible to assert that there is a relationship between Chinese medicine patterns and the clinical presentation of SAH-including its related comorbidities.

Most importantly, it is also possible to infer that patients with SAH are candidates for cardiac rehabilitation, even from the traditional Chinese medicine point-of-view. Recent systematic reviews found that Chinese medicine mind-body exercises such as *qigong*^[20] and *taijiquan*^[21] can be of benefit for patients undergoing antihypertensive

Table 1 Summary description of studies on the asserted issues linking evidence-based and Chinese medicines

Ref.	Study characteristics	Main results	Main limitation
Natural history of patterns			
Luiz <i>et al</i> ^[12]	Cross-sectional observational design Forty-three patients with hypertension grades I, II and III	Patterns were strongly or moderately associated with target-organ damage Manifestations were at most weakly associated with hemodynamic variables	Target-organ damages were not confirmed by laboratory or imagery methods Patients were under antihypertensive drug therapy
Chan <i>et al</i> ^[20]	Systematic review (8 studies) Seven randomized controlled trials and one non-randomized controlled clinical trial	Qigong improved physical symptoms in patients with coronary artery disease Qigong improved functional capacity of cardiac patients Qigong reduced blood pressure levels No adverse effects reported	Overall poor quality of most studies included in the review Study heterogeneity
Yeh <i>et al</i> ^[21]	Systematic review (26 studies) Nine randomized controlled trials, thirteen non-randomized controlled trials, and four observational studies	<i>Taijiqian</i> reduced blood pressure levels No adverse effects reported	Overall poor quality of most Chinese studies included in the review Study heterogeneity
Pulse palpation			
Luiz <i>et al</i> ^[12]	Cross-sectional observational design Forty-three patients with hypertension grades I, II and III	Frequency analysis of clinical manifestations and pulse images of patterns Most frequent pulse image: wiry pulse (52% of the cases)	Patients were under antihypertensive drug therapy
Ferreira <i>et al</i> ^[25]	Cross-sectional observational study Twenty-nine healthy subjects and twenty-three patients with hypertension grades I, II or III	Higher pulse wave velocity and lower arterial compliance of the brachial-radial artery segment in hypertension	Arterial tonometry was subjected to transducer set-up and manual positioning Patients were under antihypertensive drug therapy
Ferreira <i>et al</i> ^[26]	Cross-sectional observational study Sixty-three healthy subjects and fifty-two patients with hypertension grades I, II or III	Lower arterial compliance of the brachial-radial artery segment in hypertension Hypertrophic remodeling of medium-sized arteries in hypertension	Arterial tonometry was subjected to transducer set-up and manual positioning Patients were under antihypertensive drug therapy
Ferreira <i>et al</i> ^[27]	Cross-sectional observational study Sixty-three healthy subjects and fifty-two patients with hypertension grades I, II or III	Impaired flow-mediated vasodilation in hypertension	Arterial tonometry was subjected to transducer set-up and manual positioning Patients were under antihypertensive drug therapy
Ferreira <i>et al</i> ^[28]	Cross-sectional observational study Sixty-three healthy subjects and fifty-two patients with hypertension grades I, II or III	Increased peripheral vascular resistance immediately after ischemic occlusion Slower response to flow-mediated vasodilation	Arterial tonometry was subjected to transducer set-up and manual positioning Patients were under antihypertensive drug therapy
Lu ^[29]	Cross-sectional observational study Fifty-nine patients with hypertension grades I, II or III	Higher amplitudes for harmonics #0 (heart), #1 (liver), #3 (spleen), #4 (lung), and #6 (gallbladder) in hypertension	Poor description of the studied sample Patients were under antihypertensive drug therapy
Ferreira ^[30]	Computational simulation study Model of the radial artery during "simultaneous pressing"	Lack of correspondence between pressure pulse spectral harmonics and Chinese medicine theory of pulse palpation	No experimental data from patients with hypertension
Herbal therapy			
Xiong <i>et al</i> ^[32]	Narrative review	Herbal therapy may potentially reduce blood pressure variability, inhibit sympathetic activity, prevent target-organ damage, and improve insulin resistance	Potentially biased (selection and report bias) Some results outcome from animal studies not yet tested in humans

treatment. The benefits of *qigong* practice may include the alleviation of physical symptoms related to cardiovascular disease (CVD) (*e.g.*, 63% of the group presented relieving of coronary artery disease symptoms) and the control of blood pressure (*e.g.*, 88% of the group presented lower blood pressure levels) after 1-year practice, and the increase in functional capacity (*e.g.*, 13.7% higher six-minute walk distance after a 16-wk *qigong* training program)^[20]. Likewise, the benefits of *taijiqian* practice may include a reduction in systolic and diastolic blood pressures (3-32 mmHg and 2-18 mmHg, respectively)^[21]. However, it is

not clear whether the effects on blood pressure are due to the traditional aspects of Chinese medicine practice or to the increased physical activity itself, or both. Nevertheless, further research is necessary to determine whether Chinese medicine therapy indicated from pattern differentiation is of benefit to patients with SAH, either at secondary or tertiary level of prevention.

Pulse palpation

Clinical examination in Chinese medicine is not different from that practiced in evidence-based medicine: inspec-

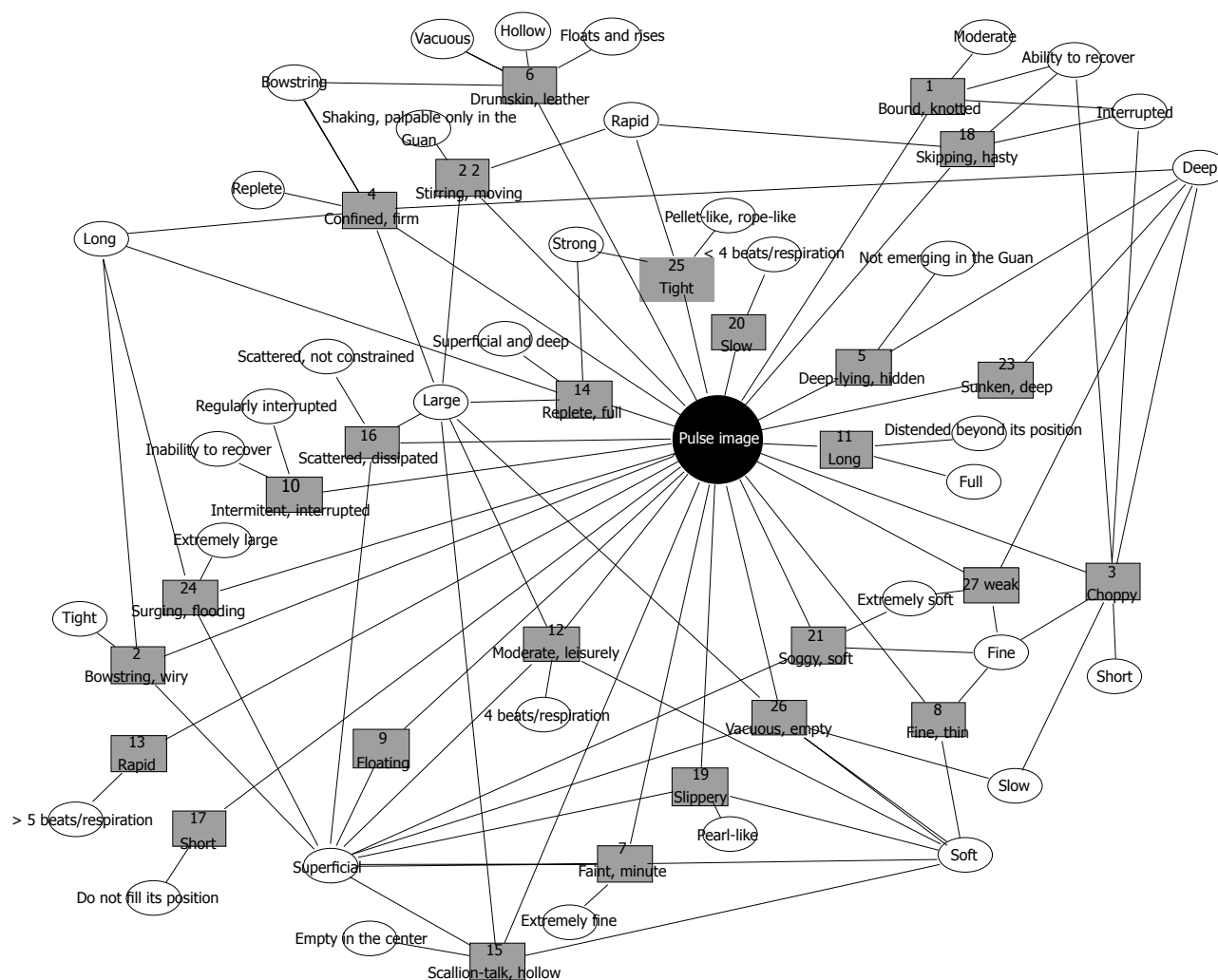


Figure 1 Pulse image network. The classic pathologic 27 pulse images (greyish, rectangular nodes) described by common attributes (whitish, ellipsoid nodes) derived from categories (frequency, rhythm, wideness, depth, and qualities). Notice that there are pulse images described by exclusive attributes, while other pulse images are described by shared attributes.

tion, auscultation and olfaction, inquiry and palpation. The most striking difference is that even today Chinese medicine health providers do not make use of any complementary exam or equipment (e.g., arterial tonometry, imaging or laboratorial data), thus relying exclusively on the subjective assessment of the five senses for confirmation or exclusion of possible patterns. Among these examinations, pulse palpation is probably the most famous and intriguing one, since antiquity until present days^[22].

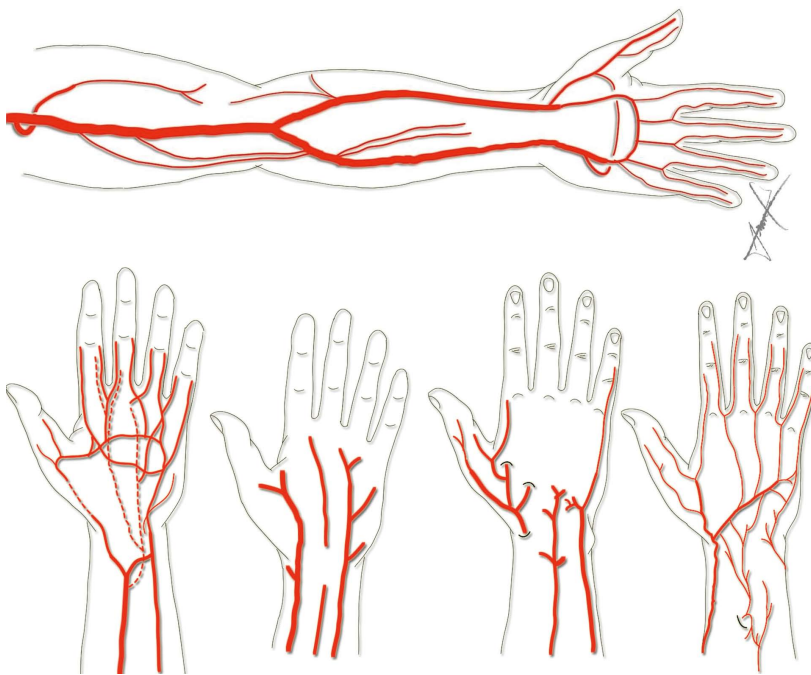
Fundamental attributes of the arterial pulse such as frequency, rhythm, wideness, and depth are shared between Chinese and evidence-based medical practices. Descriptions of abnormal pulses as palpated at either the radial or carotid artery are established for clinical diagnosis of patients with cardiovascular diseases (CDV)^[23]. Chinese medicine practitioners also make use of subjective attributes to describe their feeling of the pulse – the so-called *pulse image*^[22]. Figure 1 exhibits the network of all 27 pathological pulse images from descriptions arranged by attribute^[22] as generated by Cytoscape 3.0.0^[24]. It can be observed that there are pulse images described by

exclusive attributes (e.g., “rapid” or “short” pulse), while other pulse images are described by shared attributes (e.g., “weak” or “fine” pulse). In particular, the “deep”, “fast”, “slippery”, “strong”, “thin” and “wiry” pulse images are frequently observed in patterns related to SAH (e.g., wiry pulse = 52%, thin pulse = 25.6%, deep pulse = 7%)^[12]. Therefore, it is possible to assert that there is a relationship between the abnormal pulses and pulse images, although no evidence on this specific relationship in patients with SAH have been presented so far using quantitative pulse wave analysis.

In the last decades, pulse wave analysis using radial artery tonometry along with mathematical simulation and modeling has been used for the noninvasive assessment of both anatomic and functional status of arteries^[25]. For instance, previous studies showed that patients with SAH may present increased pulse wave velocity and decreased radial artery compliance^[25], medium-sized arteries hypertrophic remodeling^[26], and impaired flow-mediated vasodilation characterized by smaller and slower radial artery vasodilation^[27,28]. These adaptive characteristics

Table 2 Summary description of studies on the neglected issues linking evidence-based and Chinese medicines

Ref.	Study characteristics	Main results	Main limitation
Anatomical variations of vessels			
Chen <i>et al</i> ^[43]	Cross-sectional observational study One hundred healthy subjects, of normal or abnormal pulses forty-six with pancreatitis, forty-two with duodenal bulb ulcer, twenty-two with appendicitis, and third-eight with acute appendicitis	Accuracy of 82% for classification using an auto-regressive model for analysis of wrist pulse signals (blood flow signal) and a support vector machine	Ultrasound-based blood flow measurements was subjected to manual positioning and operator experience Only one position was investigated (above the styloid process) Pattern differentiation was performed (in either group) and the results were not related to Chinese medicine theory
Huang <i>et al</i> ^[44]	Cross-sectional observational study Thirty normal subjects and thirty patients with palpitation	Higher spectral harmonic energy ratio in patients	Only 10 s were evaluated at each position Palpitation was only characterized by the evidence-based medicine and no correspondence to patterns was established Pattern differentiation was performed in either group and the results were not related to Chinese medicine theory Lack of relationship between spectral harmonic energy ratio and Chinese medicine theory for pulse palpation
Hu <i>et al</i> ^[45]	Cross-sectional observational study Six normal subjects (all male)	No significant difference was observed on pulse waveform parameters obtained with single or array sensors Significant differences were observed among depths	Only one position was investigated (above the styloid process) Pattern differentiation was performed in either group and the results were not related to Chinese medicine theory

**Figure 2** Anatomical drawings on variations of the course of the radial artery. Top: Most frequent arterial pattern of the radial artery. Bottom: Examples of anatomical variations of the radial artery at the wrist.

may strongly impact on the perception of the pulse as palpated at the radial artery and are reflected in the pulse waveform signal as collected using arterial tonometry. For instance, a study showed that some spectral harmonics of the pressure pulse waveform (C0, C1, C3, C4 and C6) are higher in patients with SAH as compared to health controls^[29]. However, a more recent study^[30] failed to found a relationship between the traditional method of ‘simultaneous pressing’ for wrist pulse palpation and the spectral harmonics assigned to the respective internal organs.

Herbal therapy

In the context of therapeutics for SAH, it was recently

proposed to merge the ancient knowledge with the current one, yielding “the earlier the better for treating who and what are not yet ill”^[31]. This proposal also reflects the epidemiologic interpretation of traditional Chinese medicine while it is in agreement with the natural history of patterns related to SAH.

The use of herbs, minerals, and animal parts to compose medicinal formulas is acknowledged as the oldest therapeutic method in Chinese medicine. Considerable advances were recently achieved in the field of antihypertensive drugs, with several drug classes available for optimization of blood pressure control^[3]. However, limited efficacy for reducing blood pressure levels and side ef-

fects are among the factors that lead researchers to study other therapeutic resources, including natural compounds used in traditional medicine recipes. A large number of information about cardioprotective food is currently available and the United States Food and Drugs Administration approved and recommended some of them, even though studies are not definitive about them.

More specifically related to Chinese medicine, a recent systematic review summarized evidences in favor of Chinese herbal therapy for patients with patterns related to SAH^[32]. There are formulas that have been used widely in clinical practice for treatment of hypertension such as the *Banxia Baishu Tianma Tang* (Decoction of Pinellia ternate, Atractylodes and Gastrodia elata), *Da Chaihu Tang* (Major Bupleurum Decoction), *Liu Wei Dihuang Wan* (Pill of Rehmannia), and *Banxia Baishu Tianma Tang* (Decoction of Pinellia ternate, Atractylodes macrocephala, and Gastrodia elata). The general effects observed in previous studies include the reduction of blood pressure variability, inhibition of the activity of sympathetic nerve, blocking of the renin-angiotensin system, improvement of endothelial function and insulin resistance, and prevention of TOD^[32]. Altogether, it is possible to assert that ancient Chinese medicine practitioners were aware of the potential benefits of herbs on the cardiovascular system. Despite these whole-body effects, there are still some challenges for a large-scale usage of herbal therapy for Chinese medicine patterns related to SAH including the quality control of compounds, interaction among formula's compounds, and dose-response effects.

NEGLECTED ISSUES

In this section, the anatomical variations of vessels and comparative physiology are discussed. These issues are considered neglected because previous researches on integrative medicine ignored these aspects as related to the studies' main outcomes. Thus, these issues must be considered in future studies as factors for analysis and not as issues that could be assumed negligible. Table 2 presents summary information about the studies cited in this section.

Anatomical variation of vessels

The radial artery is classically described at the wrist as passing deep to the tendons of the anatomical snuff-box (Figure 2, top). However, variations in the arterial pattern—i.e., number and/or course of the arteries—of the upper limb have been observed frequently either in routine dissections or in clinical practice^[33] and are of both clinical and surgical significances^[34-39]. Variations in the origin and proximal course of this artery are the most common anomalies found in the forearm (Figure 2). For instance, a study with 150 routine dissections of the brachio-antebrachial arterial axis from adults cadavers and 10 from full-term fetuses found that 7 cases showed high origin of the radial artery, and were divided into 2 groups where one had the presence of a median artery (3

cases) and the other had the absence of the artery (4 cases)^[40]. Moreover, radial artery tortuosity, hypoplasia, and stenosis were observed in patients undergoing transradial coronary intervention^[41].

Chinese medicine literature states that the wrist pulse is generally felt above the styloid process of the radius and nearby proximal-distal regions in the arterial course, and that it is possible not to feel the pulse at these locations; in this case, one can feel the pulse at the external aspect of the wrist—and most importantly, it is not a sign of disease^[42]. Thus, ancient Chinese medicine scholars were aware of the existence of anatomical variations of arteries and on the distinction between pulse images resulting from normal variations and morbid patterns.

Studies have been focusing on the modernization of Chinese medicine by incorporating devices (i.e., pressure sensors) and automated methods (i.e., software tools) to acquire pressure data from the radial artery^[43-45]. However, it is intriguing that in spite of the above-cited traditional and current knowledges, none of these studies considered the anatomical variation as a confounding factor for either qualitative or quantitative pulse image analysis. Patients with hypertension are at an increased risk of presenting radial artery tortuosity^[46]. Because the geometrical characteristics of the radial artery determine the transmission of the pressure pulse waveform along the vessel^[26], it is expected that patients with SAH present pulse image characteristics due to arterial tortuosity, vascular remodeling, or both. Therefore, the anatomical variation of the radial artery cannot be neglected in future studies on pulse image analysis since it may help explain the qualitative or quantitative observed pulse image.

Comparative physiology

Recognized as the Father of western Medicine, Hippocrates (460-375 BC) and Huangdi (2695-2589 BC), reference inside the oldest known treatise of medicine in existence (the *Huangdi Neijing*) had in common in their discussions the use of acupuncture for treatment of various diseases, including coronary artery disease^[47]. Hippocrates advocated the theory of four humors—earth, air, fire and water—when trying to explain the pathogenesis of a disease, analogous to the five-phase theory of Huangdi—wood, fire, earth, metal and water. This example of comparative reasoning can be extended to all major fields of medical knowledge in Chinese and evidence-based medicines: anatomy, physiology, semiology, pathophysiology, and therapy. It is acknowledged that there are important conceptual differences between these medical practices as related to the body structures^[48], but strong similarities are empirically present at the functional level. As related to the circulatory system, Chinese medical theory also recognize its role on several functions such as the whole-body integration for distribution of substances, regulation of body temperature, and the relationship between circulation and life support^[48].

Researchers are investigating Chinese medicine searching for anatomical and/or physiological explana-

tions for the phenomena related to the safety-efficacy of interventions in the patients with SAH and other CVD^[10]. However, it is apparent that no comparative analysis have been systematically performed between Chinese and evidence-based medical theories. More specifically, it is not a matter of translation of terms from Chinese to English, but to properly transpose the interpretation of Chinese medicine knowledge to its counterpart in evidence-based medicine. For instance, such comparative reasoning may help explain: (1) the strong association observed between descriptions of TOD and patterns in patients with SAH; and (2) the similarities and dissimilarities between abnormal pulses, quantitative pulse waveform analysis, and qualitative pulse images. Therefore, it is recommended to not neglect the study of a comparative physiology between these two medical practices since it may improve our understanding on the natural history of SAH and the potential benefits of an integrated approach to patients undergoing cardiac rehabilitation programs.

DISCUSSION

Complementary and alternative medicine (CAM) are increasingly available and used for health care. A study^[49] that analyzed data on CAM use among patients with CVD found that 36% of patients with CVD had used CAM in the previous 12 mo and 10% respondents used CAM specifically for their cardiovascular conditions—among which 5% for hypertension, 2% for coronary disease, and 3% for vascular insufficiency. The same study showed that cardiac patients use mind-body therapies including deep-breathing exercises, group support, hypnosis, meditation, relaxation, *taijiquan*, *yoga*, and *shiatsu*, among others^[49]. Acupuncture, herbal Chinese medicine, moxibustion, cupping, Chinese massage, *qigong* and *taijiquan*, and dietary therapy^[50], when associated to antihypertensive medication significantly reduced systolic blood pressure (-8 mmHg) and diastolic blood pressure (-4 mmHg) with no heterogeneity detected, although given the poor methodological quality and small sample sizes of most acupuncture trials, the notion that acupuncture may lower high blood pressure remains inconclusive^[51].

In summary, the asserted issues highlighted in this paper stimulate the increasing use of Chinese medicine for health care and the continuity of research on integrative medicine in the cardiovascular field. Conversely, the neglected issues poses additional challenges that must not be overlooked in future research on this topic so that the integration of both traditional and current knowledge may be of benefit to the population with CVD.

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Alterations in cell adhesion proteins and cardiomyopathy

Jifen Li

Jifen Li, Center for Translational Medicine, Department of Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107, United States

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Correspondence to: Jifen Li, MD, PhD, Center for Translational Medicine, Department of Medicine, Jefferson Medical College, Thomas Jefferson University, Jeff Alumni Hall Suite 543, 1020 Locust St., Philadelphia, PA 19107, United States. jifen.li@jefferson.edu

Telephone: +1-215-5038262 Fax: +1-215-5035731

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Abstract

Cell adhesive junction is specialized intercellular structure composed of cell adhesion proteins. They are essential to connect adjacent heart muscle cell and make heart contraction effectively and properly. Clinical and genetic studies have revealed close relationship between cell adhesive proteins and the occurrence of various cardiomyopathies. Here we will review recent development on the disease phenotype, potential cellular and molecular mechanism related to cell adhesion molecules, with particular disease pathogenesis learned from genetic manipulated murine models.

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Key words: Cardiomyopathy; Adherens junction; Desmosome; Intercalated disc; Arrhythmia

Core tip: Cell adhesive junction is a specialized intercellular structure in the heart, and essential to maintain heart contractile function. Alterations in adhesive proteins have been found to lead to various forms of cardiomyopathy. However, the molecular and cellular mechanisms underlying heart muscle dysfunction caused by those cell adhesive molecules have not been completely understood. This review provides most re-

cent development on cellular composition of the cell adhesion proteins and their related gene mutations, disease phenotypes, potential mechanisms involved in cardiomyopathies.

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INTRODUCTION

The walls of the heart are primarily composed of elongated cardiac muscle cells, which are branched and connected each other. The site where one cardiomyocyte joins with another is called intercalated disc (ID), a specialized intercellular junctional structure found only in cardiac tissue. These structures are highly specialized and enable coordinated function of the heart mechanically to allow heart to beat^[1]. Original description of the ID identifies three structures, adherens junctions, desmosomes, and gap junctions. Recognition of the area composita and the determination of interactions between intercellular adhesion molecules, gap junctions, and ion channels suggest that the ID functions as a single unit where macromolecular complexes interact to maintain synchrony of the heart (Figure 1)^[2]. Alterations in adhesive proteins located at ID region have been found to lead to various forms of cardiomyopathy, often accompanied with life threaten arrhythmia and heart failure.

In this review, we will discuss the composition of adhesive junctional complexes, recent discovery on the relation of cell adhesion gene mutations and disease phenotypes and possible molecular mechanisms underlying cardiomyopathy.

CHARACTERIZATION OF CARDIOMYOPATHY AND RELATED GENETIC MUTATIONS

According to new proposed classification in 2008, cardio-

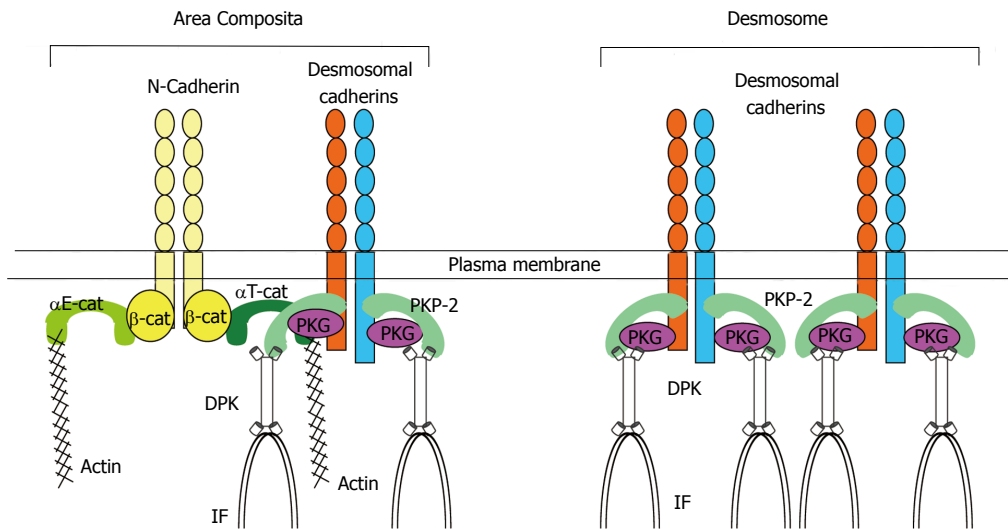


Figure 1 Components of area composita in the heart. Area composita is a mixed-type junctional structure composed of both desmosomal and adherens junctional proteins. Both α E-catenin and α T-catenin are present in the area composita at the cardiac intercalated disc. However, only α T-catenin was shown to interact directly with desmosomal protein PKP2. PKP2: Plakophilin-2; DPK: Desmoplakin; IF: Intermediate filaments.

myopathy defines as a myocardial disorder in which the heart muscle is structural and functionally abnormal^[3]. Cardiomyopathies are grouped into specific morphological and functional phenotypes, including dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), arrhythmogenic cardiomyopathy (AC), and restricted cardiomyopathy (RCM). Each phenotype is sub-classified into familial and non-familial forms^[3]. The causes of the cardiomyopathy are diverse, including genetic and spontaneous mutations of muscle proteins, hypertension, ischemia, and inflammation. Affected individuals may have a relative benign course, or develop progressive heart failure and experience sudden death, due to abnormal electrical rhythm and mechanical contractility caused by damaged heart muscle. Cardiomyopathy is most commonly diagnosed through *in vivo* imaging with either echocardiography or cardiac magnetic resonance image (MRI), which provide functional information to complement the structural changes from whole organ level.

DCM refers to enlargement of the heart, often affecting all four chambers. The prevalence of DCM is not completely known. At least 25% of patients in Western populations have evidence for familial disease with predominantly autosomal dominant inheritance. These mutations include genes encoding cytoskeletal, sarcomeric protein, Z-band, nuclear membrane and ID proteins^[4].

In contrast, HCM is characterized by increased left ventricular wall thickness, often targeting the septum that separates left ventricle from right ventricle. The prevalence of HCM is approximately 1:500 of general populations^[3,5]. Familial HCM are often caused by mutations in genes encoding cardiac sarcomeres, and often associated with congenital syndromes, inherited metabolic disorders, and neuromuscular diseases.

RCM is the most elusive, in part because the heart may appear morphological close to normal with minor increased wall thickness or modestly decreased left

ventricle ejection fraction. RCM is the least common type of cardiomyopathy and the exact prevalence of RCM is unknown. Familial RCM often occurs in autosomal dominant inheritance caused by mutations in the troponin I gene or intermediate filament desmin^[3].

AC also known as arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited arrhythmogenic disorder with estimated prevalence of 1 in 5000, and a frequent cause of sudden arrhythmic death in young^[6]. AC is defined histologically by the presence of progressive replacement of right ventricular myocardium with adipose and fibrous tissue often confined to “a triangle of dysplasia” comprising the right ventricular inflow, outflow, and apex. These pathologic abnormalities result in functional and morphological abnormalities not only in right ventricle but also in left ventricle or both, and can be present on the absence of clinically detectable structure changes. 50% of patients carry gene mutations encoding the desmosomal complexes of the ID. Majority of cases are caused by autosomal dominantly inherited mutations although autosomal recessive forms of AC are recognized^[2,3].

In practice, there is extensive overlap between these four cardiomyopathy phenotypes; for example, HCM, or AC may progress into a dilated ventricle with systolic dysfunction.

CELL ADHESION JUNCTION STRUCTURE AND COMPOSITION

Cardiac ID contains two adhesive junctions, adherens junction and desmosome, which couples cardiac muscle cells *via* actin cytoskeleton and intermediate system, respectively^[1]. The classic cadherin N-cadherin is single transmembrane protein responsible for Ca^{2+} -dependent homophilic cell-cell adhesion. The cadherin adhesive ac-

tivity is regulated by a group of proteins that bind its cytoplasmic domain, called catenins. β -catenin or γ -catenin (plakoglobin) directly binds to C-terminal region of cadherin, whereas α -catenins link cadherin/catenin complex to actin cytoskeleton^[7]. It has been shown that N-cadherin-mediated adhesion is essential for embryonic heart morphogenesis and development^[8,9].

Plakoglobin (PG) is the only ID component found in both adhesive junctions, and also functions as a signaling protein to modulate the Wnt/ β -catenin signaling pathway. PG and its homologous protein β -catenin owe 88% amino acid identity and share common protein partners^[10]. The majority of PG and β -catenin is engaged at adherens junctions and/or desmosomes. Redistribution from junction to cytosol can markedly alter their signaling activities.

There are three α -catenin subtypes in mammals: α E-catenin, α N-catenin and α T-catenin^[11]. α E-catenin is ubiquitously expressed and it is essential for early embryonic development^[12]. α N-catenin expression is restricted to neural tissue^[13]. α T-catenin is a recently identified novel member of the α -catenin family with restricted expression in testis, cardiac muscle and neurons^[14,15]. Both α T-catenin and α E-catenin are expressed in the heart and localize to the ID. α T-catenin and α E-catenin contain vinculin homology domains, and share 57% overall amino acid identity^[14,16]. Besides structural role in the AJ junction, α -catenins also play an important role in cell signaling. For example, α E-catenin has been implicated in sensing cell density in epidermis and restricting basal cell proliferation in neural progenitor cells^[17,18]. Loss of α E-catenin triggers severe epidermal hyperproliferation and tumors in mice^[17]. A role for α -catenins in regulating proliferation in the heart is currently under investigation.

Recently, a novel, exclusive type of hybrid adhering junction is identified in the mammalian heart referred to as area composita (Figure 1)^[16,19]. Immunoelectron microscopy showed that the desmosomal proteins, such as desmoplakin (DSP) are not only restricted to the classic desmosomal junctions but also detected in large adherens-like junctional structures^[19,20]. Typical components of the classic adherens junction, including N-cadherin, β -catenin was shown to co-localize with desmosomal proteins in the majority of the area composita^[19,21]. Interestingly, the area composita is not found in lower vertebrates^[22], suggesting its role in supporting the increased mechanical load on the mammalian heart by anchoring both actin and intermediate filaments over an extended junctional area of the ID. More recently, yeast two-hybrid and co-immunoprecipitation showed that α T-catenin interacts directly with desmosomal plakophilin-2 (PKP2) at area composita^[16]. However, α E-catenin lacks plakophilin-binding domain, and the interaction of α E-catenin with PKP2 is not observed in the heart^[16].

Recent studies have identified a novel ID protein, Xin. Xin is a muscle specific protein (mXin) associated with adherens junction through its interaction with β -catenin and actin cytoskeleton^[23]. The human homolog of mXin α , Cm α 1, maps to chromosomal region 3p21,

a region linked to familial DCM. However, mXin α associated mutations in human have not been identified.

Desmosome consists of desmosomal cadherins, armadillo family protein plakoglobin and plakophilin, and the plakin protein DSP^[7]. Desmosomal cadherins are transmembrane proteins and form Ca^{2+} -dependent heterotypic cell-cell adhesive interactions. In the heart, only desmoglein 2 (DSG2) and desmocollin 2 (DSC2) are expressed. Both plakoglobin and PKP2 bind cytoplasmic DSG2 and DSC2, and regulate cadherin adhesive activity and implicate in signaling. DSP links membrane desmosomal cadherins to cytoplasmic intermediate filaments^[7].

Gap junctions are intercellular channel that allow ions to travel from cell to cell and electrically couple myocytes. Six connexin molecules interact with one another to form a channel. Compared to noncardiac tissue, the ID contains extremely large connexin 43-containing gap junction plaques in the heart, reflecting its important function in electrically coupling of cardiomyocytes^[24,25].

ALTERATIONS IN CELL ADHESIVE PROTEINS AND RELATED HUMAN CARDIOMYOPATHY

Role of adherens junction-associated proteins in human cardiomyopathy and heart failure

Studies performed in humans have demonstrated that alterations and/or mutations in the ID components are associated with a spectrum of human cardiomyopathy (Table 1). Cardiac myofibril disarray is a common feature of HCM. Studies on cases with HCM reveal the ID frequently irregular or redistributed from perpendicular to parallel of myofibril^[26], with presence of decreased immunoactive signal of N-cadherin. Degenerating cardiomyocytes occasionally can be seen in HCM heart forming vacuole-like structures accompanied with strong positive staining for N-cadherin. Examination of 62 end-stage explants hearts with previous existing cardiomyopathies shows general reduction of cadherin/catenin components, often accompanied with tight junction protein claudin-5 or gap junction connexin 43 reduction^[27]. A genetic screen on the entire coding region of N-cadherin gene from 96 Japanese healthy individuals identified eight sequence polymorphisms. Three of the five single-nucleotide polymorphism has an amino acid substitution, including Ala826Thr substitution in exon 15 which is located in N-cadherin binding domain of Shc^[28]. Shc is an adaptor protein and has been shown to participate in signaling pathway that control cell growth. Although germline mutation in gene encoding N-cadherin has not been detected in the familial HCM patients^[28], these data indicate ID components may play a role in the pathogenesis of human cardiomyopathy.

Characterization of the cell adhesion protein expression in myocardial infarct rupture patients demonstrates a significantly reduced expression of α E-catenin in both total tissue level and in the ID of infarct rupture area^[29]. In contrast, other junctional components are not sig-

Table 1 Adhesive proteins-associated cardiomyopathies in human and murine models

Adhesive junctional component (Gene)	Cardiac phenotype			
	Human cardiomyopathy	Ref.	Mouse model of cardiomyopathy	Ref.
N-cadherin (CDH2)	DCM, HCM, heart failure	[26-28]	GOF: DCM, cardiac calcification LOF: DCM, ventricular arrhythmia, sudden cardiac death HET: Normal cytoarchitecture, induced arrhythmia	[42,58] [43,44] [25]
β -catenin (CTNNB1)	HCM, heart failure	[27,61]	GOF: DCM, premature death LOF: Normal cardiac function, blunt response to induced hypertrophy HET: Normal cardiac structure, reduced response to hypertrophy	[53] [50,51] [52]
Plakoglobin (JUP)	AC, Naxos disease	[30,31] [27,32,34,35]	GOF (wild-type): adipocyte accumulation, inflammation GOF (Naxos mutation): adipocyte accumulation, inflammation, cardiac dysfunction, apoptosis LOF (perinatal): early onset of cardiomyopathy, severe ventricular arrhythmia LOF (adult): dilated cardiomyopathy, apoptosis, inflammation, fibrosis HET: Aged animals with right ventricular dilation, arrhythmia	[46] [47,48] [45] [62] [49]
α T-catenin (CTNNA3)	DCM, AC	[9,36]	LOF: DCM, arrhythmia, area composita defects	[56]
α E-catenin (CTNNA1)	Post-MI ventricular rupture Heart failure	[29]	LOF: Progressive DCM, RV dilation, MI-induced ventricular rupture HET: MI-induced ventricular rupture	[54] [29]
mXin α (mXin α , Cmya1)	None	None	LOF: HCM, arrhythmia, ID defects	[23]
Desmoglein2 (DSG2)	AC	[63]	LOF: Dying cardiomyocytes with calcification, complete dissociation of intercalated discs, fibrotic replacement GOF (N266S): Sudden death, ventricular arrhythmias, cardiac dysfunction, biventricular dilatation, aneurysms GOF (N271S): Intercellular space widening, fibrosis, increased arrhythmia, lower sodium channel current density	[64] [59] [65]
Desmocollin2 (DSC2)	AC	[66-68]	None	
Plakophilin2 (PKP2)	AC	[63,69]	HET (haploinsufficiency): Impaired ventricular conduction, sodium channel dysfunction	[60]
Desmoplakin (DSP)	AC, Carvajal syndrome, heart failure	[38,41,56]	LOF: Impairs cardiac morphogenesis and leads to high embryonic lethality GOF (R283H): Apoptosis, fibrosis, lipid accumulation, ventricular enlargement and cardiac dysfunction HET: Excess adipocytes, fibrosis, increased apoptosis, cardiac dysfunction, and ventricular arrhythmias	[46,47,57] [58] [57]

DCM: Dilated cardiomyopathy; HCM: Hypertrophic cardiomyopathy; AC: Arrhythmogenic cardiomyopathy; LOF: Loss-of-function; GOF: Gain-of-function; HET: Heterozygous; ID: Intercalated disc.

nificantly changed in the injured area. This is consistent with the observation that α E-catenin heterozygous mice exhibit ventricular rupture post myocardial infarction^[29]. These results suggest that patients with an intrinsic defect in their cell adhesion complex may predispose myocardial rupture after experiencing ischemic stress.

Plakoglobin (PG) encoded by the *JUP* gene is the first component of the desmosome to be implicated in the pathogenesis of AC^[30]. Studies of individuals from the Greek island of Naxos identified an autosomal recessive form of AC with palmoplantar keratoderma and woolly hair referred to as Naxos disease. Gene sequencing revealed a homozygous 2-bp deletion (2157-2158delGT) in the *JUP* gene in affected individuals^[31]. A study of a German family reported the first dominantly inherited *JUP* gene mutation (S39_K40insS) to cause AC without cutaneous abnormalities^[32]. Both mutant forms of PG failed to localize properly at the ID, and the junctional components DSP and Cx43 were significantly reduced at the ID in these patients. Ultrastructural investigation showed ID remodeling with mislocalization and a decreased number of desmosomes^[6,32]. Importantly, a reduced immunoreactive PG signal at the ID is a consistent feature in patients with dominant mutations in a variety of desmosomal

genes, making PG a potential diagnostic tool for AC in affected individuals^[33]. Recently, additional mutations in the *JUP* gene have been identified, including homozygous Q539X, S24X and missense 468G > A mutations. These young patients showed skin but not heart abnormalities although further examination will be required to rule out no cardiac phenotype^[34, 35].

The human α T-catenin gene, *CTNNA3*, has been mapped to chromosome 10q21, a region linked to autosomal dominant familial DCM^[9]. Although genetic screening has not detected any DCM-linked *CTNNA3* mutations to date, *CTNNA3* is considered a candidate gene and may be the potential cause of this disease^[9]. Utilizing denaturing high-performance liquid chromatography and direct sequencing, for the first time two gene mutations in α T-catenin have been identified from 76 AC patients who did not carry any mutations in the desmosomal genes commonly mutated in AC^[36]. Mutation c.281T > A (p.V94D) is located in N-terminal β -catenin binding domains of α T-catenin. Over expression p.V94D mutant in heart muscle cells shows diminished interaction of α T-catenin and β -catenin, whereas mutation c.2293_2295delTTG (p.del765L) at C-terminal of α T-catenin results in deletion of leucine in position 765 of

α T-catenin protein. The p.del765L mutant protein shows a much stronger dimerization potential and forms aggregates in a nonmuscle cell line. Whether the area composita assembly and function is perturbed in CTNNA3 mutant AC patient heart remains unclear. Nevertheless, this is the first report on the involvement of area composita gene in AC and may suggest the pathogenesis of this disease extend beyond desmosomes. Clinically, the affected individuals showed severe right ventricle dilation, intramural and epicardial fibrosis in left ventricle, reduced right ventricular ejection fraction, and sustained ventricular tachycardia with left bundle-branch block^[36]. Since the frequency of CTNNA3 mutations in AC patients is not rare, systematic screening for this gene should be considered to improve the clinical management of AC families.

Role of desmosome-associated proteins and human arrhythmic cardiomyopathy

To date, human genetics studies have identified 12 independent loci and 8 disease genes for AC^[2]. Five of 8 causative genes encode major components of the cardiac desmosomes, namely plakoglobin (JUP), DSP, PKP2, DSG2, and desmocollin-2 (DSC2). Up to 50% of AC probands harbor a mutation in 1 of these genes^[2,37]. Mutations in desmosomal genes with recessive and dominant patterns of inheritance are associated with cutaneous disease, cardiac disease, or both. Mutations in desmosomal cadherins DSG2 and DSC2 account for 10% of cases of AC^[38,39]. The phenotype includes characteristic histological and clinical feature of AC, with prominent left ventricular involvement in many cases. Heterozygous PKP2 mutations account for the highest proportion of cases, and the reported prevalence is about 43% among US studies^[38, 40]. DSP is the first gene to be implicated in autosomal dominant AC mutations. In 2002, a missense mutation (S299R) in DSP was identified in an Italian AC family. The patients show classic AC phenotype with arrhythmia of right ventricular origin with instances of ventricular fibrillation and sudden cardiac death^[41]. Interestingly, recent genetic analysis has identified AC patients with mutations in more than one desmosomal gene supporting a multigenic etiology to this disease (Table 1).

ALTERATIONS IN CELL ADHESION PROTEINS IN GENETICALLY MANIPULATED ANIMAL MODELS OF CARDIOMYOPATHY

Despite human genetics studies have been successful in identifying disease-causing genes, multiple interacting factors, including genetic background; various environmental stimuli (hemodynamic stress, inflammation, and metabolism) can influence the ultimate clinical outcome and diagnosis. In past decades, genetically engineered mouse models have been widely used and provided invaluable resources for understanding pathogenesis of cardiomyopathy (Table 1).

Role of adherens junction-associated proteins in animal models of cardiomyopathy

N-cadherin is the only classical cadherin expressed in the myocardium, and plays a key role in maintaining cardiac structure integrity. Ectopic expression of epithelial cadherin (E-cadherin) in the myocardium causes early onset of HCM, cardiac calcification, and increased mortality. Overexpression of N-cadherin in adult mouse heart leads to dilation of the left ventricle, redistribution of β -catenin, Cx43 and upregulation of pathological marker atrial natriuretic factor^[42]. Induced deletion of N-cadherin specifically in the adult mouse heart (N-cad CKO) results in disassembly of the ID structure, dilation of ventricular and atrial chambers, reduced wall thickness, and fibrosis^[43,44]. Cardiac-gated MRI image data demonstrate significantly larger left ventricular end-diastolic volume and end-systolic volume in the N-cad CKO group. Both ejection fraction and cardiac output are significantly reduced. These results are consistent with a decrease in force transmission due to loss of the cadherin/catenin adhesion complex at the ID. Using miniaturized electrocardiogram telemetry transmitters implanted in N-cad CKO mice, abrupt onset of spontaneous ventricular tachycardia was observed immediately prior to sudden death. The lethal arrhythmias were associated with decrease gap junction protein Cx43 and slow electrical conduction in the N-cad CKO mice. Relocalization and/or loss of Cx43 from the ID are often observed in human diseased myocardium^[1]. In contrast, animals with half the normal level of N-cadherin show the normal heart histology and normal life span. However, the heterozygous mice exhibit an increased susceptibility to arrhythmia induced by electrical stimuli^[25]. These mouse models demonstrate the critical role of N-cadherin in maintaining the ID structure, and suggest perturbation of the adhesive junctional complex may underlie the pathogenesis of cardiomyopathy.

Several groups have generated animal models of AC by manipulating plakoglobin expression in mice. Adult mice with inducible cardiac restricted deletion (CKO) of the *JUP* gene exhibit progressive loss of cardiac myocytes, DCM and cardiac dysfunction. Consistent with altered desmosome ultrastructure in plakoglobin CKO hearts, expression of desmosomal proteins are decreased at the ID. Focal areas of myocyte loss and replacement by fibrous tissue, along with patchy inflammatory infiltrates, are revealed in the myocardium of PG CKO. Animals with perinatal myocardial deletion of *JUP* gene exhibit early onset cardiomyopathy and severe ventricular arrhythmias^[45]. Deletion of *JUP* in the developing heart before maturation of the ID likely explains the more severe phenotype compared to deletion in the adult heart^[45]. Cardiomyopathy is also observed in mice overexpressing either wild-type (WT)^[46] or truncated PG (*i.e.*, Naxos)^[47,48] in the heart. In both models, PG accumulates in the nuclei of the cardiomyocytes. The molecular mechanisms involve activation of Hippo signal pathway, inhibition of Wnt/ β -catenin target genes and enhanced adipocyte gene expression in the mutant PG mouse heart. Interestingly, it has been reported that heterozygous PG-null mice ex-

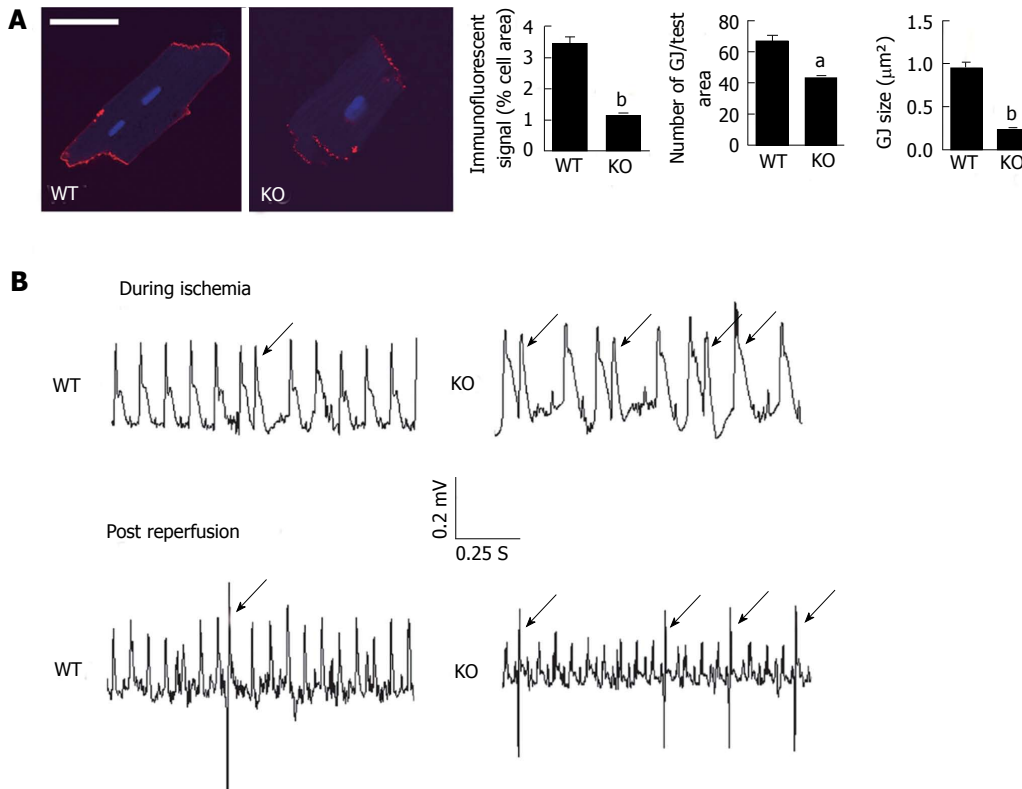


Figure 2 Loss of α T-catenin in the mouse heart leads to reduced expression of Cx43 and ventricular arrhythmia following acute ischemic injury. A: Adult cardiomyocytes isolated from wildtype (WT) and α T-catenin knockout (KO) hearts were immunostained for Cx43. Ten cardiomyocytes from each animal were examined for five or more contiguous pixels of high signal intensity. The amount of specific immunoreactive signal at intercalated disc (ID) for Cx43, the number of Cx43-containing plaques (gap junction, GJ) and their size (GJ size) were quantified and are shown in the panel at right. Scale bar; 50 μ m. The error bars represent the s.e.m. ^a $P < 0.05$; ^b $P < 0.01$; B: Representative telemetry ECGs of different patterns of premature ventricular contractions (PVCs, arrow) during ischemia-reperfusion (I-R) injury in WT and α T-catenin KO mice. Mice from WT and α T-catenin KO were subjected to ligation of the left anterior descending artery for 30 min and 7 d reperfusion. A miniaturized telemetry ECG transmitter was implanted before I-R.

hibit altered right ventricular contractility and arrhythmia without affecting myocardial structure at 10 mo of the age. Endurance exercise (*e.g.*, daily swimming) exacerbates disease progression in these mice^[49] suggesting endurance exercise can enhance disease progression among the people suffering AC.

In contrast to PG, β -catenin is not required for maintaining the mechanical junctions in adult myocardium in physiologic conditions. The upregulation of PG and its ability to substitute for β -catenin in adherens junction are responsible for the lack of ID defects in β -catenin knockout mice^[50]. However, compared to wild-type, β -catenin mutant mice are unable to respond to hypertrophy induced by hemodynamic stress, indicating β -catenin signaling is essential to pathological hypertrophic growth of cardiomyocytes^[51,52]. In comparison, mice with overexpression of non-degradable or active form of β -catenin develop DCM, and premature death^[53]. These data suggest that both localization and cellular signaling changes mediated by β -catenin can cause abnormal cardiac function as well as cardiomyopathy.

Alpha-catenins are key cytoplasmic molecules thought to be indispensable for maintenance of tissue morphogenesis. α E-catenin is ubiquitously expressed in all tissue. Ablation of α E-catenin expression specifically in the mouse heart results in progressive DCM, defects in right

ventricle, and reduced expression of cytoskeleton protein, vinculin in ID region. Similar to the human ventricular rupture patients mentioned above, these mice exhibit increased susceptibility to ventricular free wall rupture after myocardial infarction^[54]. α T-catenin is a recently identified novel member of the α -catenin family with restricted expression in heart and also the only α -catenin in the adherens junction that interacts with the desmosomal protein PKP2 (Figure 1)^[14-16,55]. Germline deletion of α T-catenin in mice alters PKP2 distribution without affecting other junctional components of the area composita. Phenotypically, these mice exhibit early onset DCM, cardiac dysfunction, and gap junction remodeling. Our study suggested that disruption of the area composita in the α T-catenin KO hearts weakens the actin and the desmin cytoskeletal networks that results in a reorganization of the cytoskeleton and leads to alteration of expression and cellular distribution of Cx43 and gap junction remodeling (Figure 2)^[56]. Furthermore, the diminished levels of gap junctional Cx43 in the ID of α T-catenin-ablated cardiomyocytes, as well as the reduced number and size of Cx43-containing gap junction plaques in α T-catenin-KO cardiomyocytes *in vitro* and *in vivo*, may lead to an increased incidence of arrhythmias. In response to acute ischemic injury, the α T-catenin mutant mice exhibit increased ventricular arrhythmia^[56]. Importantly,

although reperfusion is essential to prevent irreversible cellular injury and preserve ventricular function, reperfusion and the attendant recovery from ischemia can cause ventricular arrhythmias, cellular injury, and SCD. In this regard, it is important to emphasize the increased susceptibility to ventricular arrhythmia observed after the first 24 h of reperfusion in α T-catenin KO animals in comparison with WT (Figure 2)^[56]. Taken together, these data demonstrate that alterations in either α E- or α T-catenin can cause DCM. Because of the unique interaction with desmosomal PKP2, α T-catenin may play more important role than α E-catenin in maintaining area composita structure and function. The identification of α T-catenin not α E-catenin mutations in AC patients provides further evidence for a unique role of α T-catenin in the pathogenesis of AC.

In the mouse, there are two homologous genes of mXin α and mXin β . Mice with germline deletion of mXin α exhibit HCM accompanied by disruption of the ID. Prolonged QT interval is detected from ex vivo isolated mXin α mutant mouse heart, suggesting loss of mXin α perturbing conduction system of the cardiac muscle cells^[23].

Role of desmosome-associated proteins in animal models of cardiomyopathy

DSP is a major desmosomal component, and indispensable for the linkage of the desmosomal cadherins to cytoskeletal filament network. Mice with cardiac restricted deletion of DSP in perinatal heart exhibit a high incidence of embryonic lethality with malformation of heart structure. In contrast, heterozygous DSP knockout mice are viable and display AC-like phenotype^[57]. Histology analysis shows enlarged ventricles, poorly organized myocytes with large area of fibrosis, and excess accumulation of fat droplet in the myocardium. Echocardiography demonstrates the thinning wall, increased end-diastolic and end-systolic dimension and reduced systolic function. Further study demonstrates that DSP deficiency results in nuclear translocation of plakoglobin and reduction of β -catenin-mediated Wnt signaling thus enhancing adipogenic gene expression^[57]. Transgenic mice with cardiac-restricted overexpression of the AC-associated DSP mutation (R283H) exhibit increased cardiomyocyte apoptosis, cardiac fibrosis, and lipid accumulation, along with ventricular enlargement and cardiac dysfunction in both ventricles^[58].

Recently a transgenic mouse model overexpressing the human AC-associated mutation N266S in DSG2 has been generated. The DSG2-N266S transgene mice exhibit a biventricular cardiomyopathy with aneurysms, ventricular arrhythmias, and sudden death. Histological study demonstrates pronounced myocardial damage, coagulative necrosis, massive neutrophil infiltrate, and calcification^[59].

Heterozygous mutations in PKP2 are the most common mutations in AC patients. However, transgenic mice with overexpression of PKP2 AC-associated mutations have not been generated. Constitutive knockout of PKP2

in mice leads to embryonic lethality due to ventricular free wall rupture^[12]. Interestingly, heterozygous PKP2 mice without histological or gross anatomical abnormalities in hearts exhibit impaired ventricular conduction, altered electrocardiographic parameters and arrhythmic death when treated with sodium channel blocker^[60]. These results suggest a possible cross talk between desmosome and sodium channel complex, and sodium current dysfunction may contribute to arrhythmogenesis in PKP2-deficient hearts.

CONCLUSION

Genetic mutations account for a significant percentage of cardiomyopathies, and are a leading cause of congestive heart failure. Thanks to advanced study on structure and function of human genes and widely available genetic screening for mutated genes, genetic cardiomyopathy is now more commonly diagnosed. The primary role of adhesive junctional complexes is providing mechanical attachment between muscle cells by linking cellular membrane to cytoskeleton filaments. Mutations in genes encoding adherens junctional or desmosomal proteins disrupt either cell-cell adhesion, or membrane-actin/intermediate filament interaction, or both, thus affecting contractility and cell-cell communication. With respect to the latter, decrease in conduction velocity can lead to re-entry, causing ventricular arrhythmia and sudden cardiac death. The underlying mechanisms may include adhesion proteins influence connexon trafficking, channel assembly, and/or stability at the ID. Reduced amount and organization of Cx43-containing gap junction plaques likely play a fundamental role in the increased incidence of arrhythmias. Moreover, perturbation of the normal cellular distribution of junctional proteins between the membrane versus the cytosol may alter signaling pathways, such as pathogenic activation of the Hippo pathway, suppression of the canonical Wnt signaling, leading to enhanced cell death, replacement of fibrotic adipocyte, and cardiac dysfunction.

Treatment of cardiomyopathy depends on the etiology, the severity of symptoms, complications, and age of the patient. Treatment may include lifestyle changes, medicines, surgery, and implanted devices to correct arrhythmias. Because of the crucial role of adhesive junctional complexes in the pathogenesis of cardiomyopathy, identifying specific protein interactions mediated by cell adhesive proteins may provide novel therapeutic strategies to prevent, attenuate and possibly reverse the disease phenotype.

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Autoantibodies to apolipoprotein A-1 as a biomarker of cardiovascular autoimmunity

Nicolas Vuilleumier, Fabrizio Montecucco, Oliver Hartley

Nicolas Vuilleumier, Fabrizio Montecucco, Department of Genetics and Laboratory Medicine, Geneva University Hospitals, 1211 Geneva, Switzerland

Nicolas Vuilleumier, Fabrizio Montecucco, Department of Human Protein Sciences, Faculty of Medicine, 1211 Geneva, Switzerland

Fabrizio Montecucco, Department of Internal Medicine, Foundation for Medical Researches, Faculty of Medicine, Geneva 1211, Switzerland

Oliver Hartley, Department of Immunology and Pathology, Faculty of Medicine, 1211 Geneva, Switzerland

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Correspondence to: Dr. Nicolas Vuilleumier, MD, PD, Head of Laboratory Medicine Division, Department of Genetics and Laboratory Medicine, Geneva University Hospitals, 4 rue Gabrielle-Perret-Gentil, 1211 Geneva, Switzerland. nicolas.vuilleumier@hcuge.ch

Telephone: +41-22-3729150 Fax: +41-22-3827245

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Abstract

Immune-driven inflammation plays an important part in atherogenesis and is therefore believed to be key to the development of cardiovascular disease (CVD), which is currently the leading cause of death in the Western world. By fulfilling some of the Koch postulates, atherogenesis has even been proposed to be considered as an autoimmune disease, raising the hope that CVD could be prevented by immunomodulation. Nevertheless, the role of the immune system and autoimmune reactions in atherosclerosis appear to be a double edged sword, with both pro-atherogenic and anti-atherogenic attributes. Hence, if immunomodulation is to become a therapeutic option for atherosclerosis and CVD, it will

be crucial to correctly identify patients who might benefit from targeted suppression of deleterious autoimmune responses. This could be achieved, for example, by the detection of disease-associated autoantibodies. In this work, we will review the currently available clinical, *in vitro*, and animal studies dedicated to autoantibodies against apolipoprotein A-1 (anti-apoA-1 IgG), the major proteic fraction of high density lipoprotein. Current clinical studies indicate that high levels of anti-apoA-1 IgG are associated with a worse cardiovascular prognosis. In addition, *in vitro* and animal studies indicate a pro-inflammatory and pro-atherogenic role, supporting the hypothesis that these autoantibodies may play a direct causal role in CVD, and furthermore that they could potentially represent a therapeutic target for CVD in the future.

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Key words: Autoantibodies; Cardiovascular disease; Atherosclerosis; Apolipoprotein A-1; Autoimmunity; Biomarkers

Core tip: This review provides a comprehensive and critical analysis of the most recent basic research articles and clinical trials on the role of autoantibodies to apolipoprotein A-1 as biomarkers and potential mediators of cardiovascular diseases (CVD). Evidence from both *in vitro* and *in vivo* studies showed that anti-apolipoprotein A-1 IgG might have critical pro-atherosclerotic activities by activating immune cells to release pro-inflammatory mediators and proteases. In addition, these autoantibodies might increase heart rate and arrhythmias both in humans and animal models. These studies suggest a causal role of anti-apolipoprotein A-1 immunoglobulins of G class in CVD, indicating that those autoantibodies could potentially represent an emerging therapeutic target to better fight CVD.

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INTRODUCTION

Current epidemiology of cardiovascular diseases and preventive strategies

Despite increasing public awareness and major therapeutic progress, cardiovascular disease (CVD) remains the leading cause of morbidity and mortality worldwide. In the United States, CVD prevalence in the general population is expected to reach 40%, with direct related costs set to reach 800 billion dollars per year in the next two decades^[1].

In Europe, CVD causes 47% of all deaths (Figure 1), accounting for 4 million fatalities each year, and costing 196 billion euros a year. Roughly half of these costs (54%) have been attributed to direct health care costs, and the other half (46%) to indirect losses (Heart Network: www.ehnheart.org).

Because the disease progresses asymptotically, the first indication that an individual has atherosclerosis is often a severe cardiovascular event. According to statistics obtained in the United States during the last two decades, the first indicator of atherosclerosis for 30%-50% of patients was an acute, and in many cases fatal, myocardial infarction (MI)^[2]. Current guidelines address this problem by identifying high-risk individuals according to the cumulative presence of different Framingham risk factors (smoking, obesity, diabetes, dyslipidemia, and hypertension), with the decision to go forward into preventive treatment made according to the estimated risk. Based upon these clinically-based cardiovascular (CV) risk stratification tools, individuals identified as at-risk for atherosclerosis and CVD are subjected to treatment that directly addresses the established risk factors, combining lifestyle modification (*e.g.*, smoking, exercise, diet) with anti-platelet therapy (aspirin), and medication to reduce both blood pressure (anti-hypertensive agents) and levels of circulating cholesterol (statins).

While this strategy has undoubtedly made some impact, current CV risk stratification tools only have the power to segregate very high-risk individuals from very low-risk individuals, and lack sensitivity and specificity in persons deemed to be at intermediate risk^[3]. As a consequence, up to 60% of CV events occur in primary prevention (*i.e.*, in patients with asymptomatic CVD), affecting subjects deemed at low or intermediate risk of CVD (false negative)^[4,5]. At the same time, other patients are unnecessarily given lifelong prevention medication (false positives) (reviewed in^[2,6]).

For this reason, strong calls have been made to exploit existing knowledge and technology to improve the sensitivity and specificity of risk stratification approaches used to guide preventive therapy^[2]. To be effective as public health measures, new approaches would have to

be not only sensitive and specific, but also low-cost, non-invasive and adaptable to scale-up and commercialization for widespread use^[2,6]. While solutions involving imaging technologies such as ultrasound, chest computed tomography (CT) and magnetic resonance imaging have been proposed^[2], their implementation at population level in primary care is currently difficult to envisage mainly for economic reasons, and also because of health hazards related to radiation exposure.

As a more viable alternative strategy with respect to costs and health issues, much attention has been drawn to CV biomarkers that allow, on the basis of a simple blood sample measurement, to quantify either the amount of myocardial necrosis, such as cardiac troponins^[7], the degree of myocardial stretch, such as natriuretic peptides^[8], or the amount of systemic inflammation, such as high sensitive C-reactive protein (hs-CRP)^[9], to only quote the “usual suspects” in the field. The complete list of candidate CV biomarkers is much longer, reflecting the numerous studies published in the field (Figure 2), but only a few of these candidates, notably those shown to be causally involved in the disease, are likely to make their way into clinical practice. For this reason it is hoped that improved knowledge of the pathogenesis of atherosclerosis will lead to the identification and validation of biomarkers for atherosclerosis and CVD, enabling the development of new risk stratification approaches^[6].

Pathogenesis of atherosclerosis and cardiovascular disease

CVD is causally linked to atherosclerosis, the swelling of artery walls due to the formation of plaque lesions. Plaques are made up of leukocytes, smooth muscle cells and lipid deposits, with the surface of the plaque in contact with the arterial lumen covered with a fibrous connective tissue cap. Although atherosclerosis accumulates gradually and asymptotically from childhood, it is accelerated by a number of established risk factors, including Framingham risk factors. Atherosclerotic plaques may remain stable as they grow, gradually reducing arterial blood flow as the lumen becomes increasingly obstructed, or may become prone to rupture. When plaque rupture occurs, the highly thrombogenic interior of the plaque is revealed, leading to atherothrombosis. The resulting ischemia is what causes CVD morbidity and mortality. Depending on the location of the affected artery the outcome can be myocardial infarction, stroke, or peripheral artery disease^[4].

Atherosclerosis as an immune-mediated disease

Evidence linking high blood cholesterol to atherosclerosis, together with the presence of lipid deposits within atherosclerotic plaques led to the prevailing view that atherosclerosis was a lipid-related disease. This view was held until the 1990's, when a series of discoveries led to a paradigm shift in the understanding of atherosclerosis, shifting emphasis from lipid metabolism and transport to inflammation^[10-12]. Inflammatory responses are now believed to underlie all of the key steps in atherosclerotic

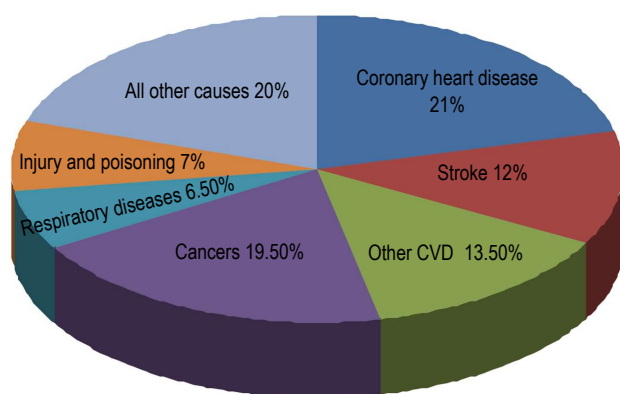


Figure 1 Deaths by cause in Europe for the latest available year, and by gender. Adapted from European Heart Network (www.ehnheart.org). CVD: cardiovascular disease.

pathogenesis, from the initial modification of healthy arterial endothelium to thrombus formation at the site of plaque rupture.

According to this current paradigm (reviewed in^[2,10-12]), atherosclerosis is initiated by inflammatory activation of arterial wall endothelial cells, allowing adhesion of circulating leukocytes. Expression of inflammatory chemokines leads to the migration of these leukocytes, predominantly circulating monocytes, across the endothelium and into the tunica intima. At this site the monocytes mature, acquiring a macrophage phenotype and the capacity to ingest native and modified low-density lipoprotein (LDL) particles that exit the blood and permeate the activated arterial endothelium. Following extensive lipid ingestion, these macrophages become “foam cells”, which are the main constituents of an early atherosclerotic lesion. Foam cells release a broad range of cytokines and serve to amplify the inflammatory response, as well as inducing the proliferation of resident smooth muscle cells and promoting local angiogenesis. Chronic inflammation leads to the formation of an advanced atherosclerotic plaque, comprising a mass of foam cells surrounding a “necrotic core” of lipids released by dead and dying cells, capped by a fibrous layer made up of smooth muscle cells and extracellular matrix. Inflammatory responses also play a key role in atherothrombosis, which is recognized to account for up to 80% of acute CV manifestations^[13]. Inflammation influences the local extracellular matrix composition through a complex interplay between different matrix-metalloproteinases (MMPs) determining the propensity of the fibrous cap to rupture^[14-17]. Furthermore, a pro-inflammatory micro-environment also promotes thrombus formation *via* the activation of coagulation factors, leading to acute vessel occlusion^[6].

Detailed analysis of the content of atherosclerotic plaques, together with the advent of a wide range of genetically modified mouse strains, has enabled further elucidation of the inflammatory pathogenesis of atherosclerosis^[18]. The identification of autoantibodies as well as autoreactive T cells in atherosclerotic plaques^[19], and the

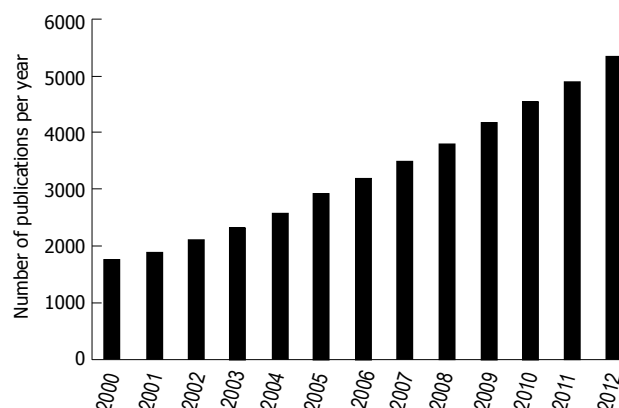


Figure 2 Annual evolution of publications on cardiac biomarkers since 2000. This graphic represents the number of publications per year indexed and retrieved in Pubmed between 2000 and 2012 when the key words “cardiovascular biomarkers” are entered. Entry date: 22nd of January 2014.

correlation established in clinical studies between their detection and disease severity provided a clear indication that adaptive immunity plays a role in atherosclerosis (reviewed in^[20]). This role was underlined in a number of studies in which ApoE^{-/-} knockout mice, which are predisposed to hypercholesterolemia and atherosclerosis, were crossed with different mouse strains deficient in specific arms of the adaptive immune system. These studies revealed a key pro-atherogenic role for the Th1 subset of CD4 T cells, and an anti-atherogenic role for the regulatory T cell subset (reviewed in^[20]), as well as both pro- and anti-atherogenic roles for different B cell subsets^[21]. In addition, they highlighted the importance in atherogenesis of signaling through pattern recognition receptors (PRR) of the innate immune system, such as Toll-like receptors (TLR) (reviewed in^[18,20]).

Atherosclerosis as an autoimmune disease?

Grounded on the fact that atherogenesis fulfills several of “Koch” postulates (Table 1), atherosclerosis has even been proposed to be of autoimmune etiology^[22,23]. This hypothesis is based on the following evidence. Firstly, atherosclerotic plaques are infiltrated by both T cells and antibodies specific for various autoantigens^[20], patients suffering from autoimmune disease, such as systemic lupus erythematosus (SLE), anti-phospholipid syndrome (APS) and rheumatoid arthritis (RA) display an increased CV risk, independently of traditional CV risk factors^[24-26]. Secondly, as reviewed elsewhere, in patients without autoimmune diseases, but established CVD, levels of antibodies directed against various and numerous endogenous epitopes, such as modified LDL, heat-shock proteins (HSP), and cardiolipin, have been shown to independently predict CV outcome^[27]. Thirdly, *in vivo* and *in vitro* evidence demonstrated that some autoantibodies might directly influence atherogenesis and atherosclerotic plaque vulnerability, mostly by activating innate immune receptors, thereby supporting a causal role of humoral autoimmunity in atherosclerosis^[28-31].

Nevertheless, the relationship between autoantibodies

Table 1 Koch posultates applied to the role of autoimmunity in atherosclerosis

Basic Koch postulates	Koch postulates transposed to the role of autoimmunity in atherosclerosis	Koch postulates met ?
Pathogens must be detected in the diseased host at every stage of the disease	Autoantibodies and auto-reactive T cells can be detected in atherosclerotic plaques and serum of patients in primary or secondary prevention of CVD	Yes
Pathogens must be isolated from the diseased host and grown in culture	Autoreactive T-cells can be isolated and cultivated from diseased host presenting experimental atherosclerosis	Yes
When inoculated in healthy animals, the pathogens from pure culture must induce the disease	Passive or active immunization drastically affect the course of atherogenesis in animal models	Yes
The pathogen must be re-isolated from the diseased animal and must correspond to the primary pathogen in pure culture	Protective autoantibodies of expected specificity can be isolated from animals exposed to active immunization	Partly

To establish a causality link between a microorganism and an infection, the four Koch postulates must be fulfilled. When applied to the role of autoimmunity in atherosclerosis, the Koch postulates support a causal role between autoimmunity, atherosclerosis and cardiovascular disease (CVD). Adapted from references^[20-28].

and CVD is debated, because some of them have been shown to be anti-atherogenic, while others act as pro-atherogenic molecules^[27,28]. The reason for such duality is still elusive and will not be further discussed in the present work.

Another unresolved question concerns the mechanisms by which tolerance is broken to generate autoimmunity. Certain lines of evidence point to pathogen molecular mimicry, *i.e.*, cross-reactivity between microbial antigens and components of host structures, including modified LDL and HSP^[32,33]. In addition, modification of proteins by oxidation can generate new epitopes that are recognized as non-self by the adaptive immune system^[32]. However, the presence of a non-self-epitope is not normally sufficient to drive an autoimmune response, since in order to effectively prime T cells, antigen presenting cells must concomitantly receive “danger signals” through their PRR. In the case of pathogen molecular mimicry, the PRR ligands are provided by the pathogen in the form of pathogen-associated molecular patterns (PAMPs). In the absence of a pathogen, “sterile inflammation” can be induced when antigen presenting cells are stimulated *via* their PRR by an analogous set of structures called damage-associated molecular patterns (DAMPs), which are typically released by stressed or necrotic cells^[31-33].

Hence, both pathogen molecular mimicry, as a consequence of infection with, *e.g.*, *Chlamydia pneumoniae* or *Helicobacter pylori*, or DAMP-mediated sterile inflammation represent mechanisms by which autoantibodies targeting antigens implicated in atherosclerosis can emerge (reviewed in^[12]).

Autoantibodies as CV risk stratification tools?

As mentioned previously, there is a clear need for new biomarkers to improve current CV risk stratification^[6,34]. Driven by the paradigm shift of atherogenesis moving from a lipid-centered to inflammatory-centered etiology, the quest for new potential cardiovascular risk markers to better assess global cardiovascular vulnerability was principally oriented on inflammatory biomarkers, including autoantibodies^[27-29].

Among the advantages identified for some autoanti-

bodies is that they meet the current benchmark specifications requested for novel CV biomarkers^[35,36]. Firstly, their association with CV outcomes has not only been shown to be independent of traditional CV risk factors (reviewed in^[27,28]), but could also provide incremental predictive information over current CV risk stratification tools. Secondly, the stability provided by their long half-life place them as good candidates for long-term prognosis when compared to biomarkers with a shorter half-life. Thirdly, their measurement is typically simple, accurate, robust, and achievable at moderate costs.

Autoantibodies as potential therapeutic targets?

Providing that some autoantibodies have been shown to modulate atherogenic processes in antagonistic ways (reviewed in^[27,28]), attempts to induce atheroprotective immunity through active immunization raised the hope that vaccination against different specific antigens (wide variety of modified LDL, HSP, *etc.*) could lead to lifelong protection against atherosclerosis and CVD. This hypothesis is currently under active investigation in humans^[37,38].

On the other hand, neutralizing the deleterious effects of pro-atherogenic autoantibodies represents another interesting therapeutic modality which could currently be achieved through passive immunization with intravenous immunoglobulins (IVIG). In this respect, data concerning IVIG administration in humans after MI yield rather contradictory results^[38,39], and although data restricted to animal models do support an anti-atherogenic role of IVIG^[40-43], the costs related to IVIG therapy may well prohibit widespread administration of IVIG to all MI or CVD patients in the long-term, even if it proves to be effective. One solution might be to identify specific autoantibodies that could then be selectively neutralized by anti-idiotypic molecules rather than IVIG. Accordingly, an approach based on the detection of specific autoantibodies would enable the identification of a subset of CVD patients that could benefit either from immunomodulation (passive or active immunization) or from a specific mimetic peptide-based therapy. Such a strategy could represent an affordable step forward toward personalized medicine in the field of CVD, allowing a more targeted therapeutic intervention.

HIGH-DENSITY LIPOPROTEIN, APOLIPOPROTEIN A-1, AND ITS RELATED AUTOANTIBODIES

Human apolipoprotein A-1 (apoA-1) is a 28-kD protein with 243 amino acid residues encoded by the apolipoprotein multigene superfamily located on chromosome 11q23^[44]. The protein is synthesized as a 24 amino-acid-longer prepro sequence of apoA-1, primarily by hepatocytes in the liver and also by enterocytes. Mature apoA-1 constitutes the principal protein fraction of high density lipoprotein (HDL) whose protective role in the CV system derives, to a great extent, from the inverse association of HDL-cholesterol and apoA-1 plasma concentrations with the risk of myocardial infarction. The atheroprotective role of HDL in the cardiovascular system has been attributed to the pleiotropic effects of HDL, including reverse cholesterol transport from resident arterial wall macrophages to the biliary tract for elimination, vasodilatation, anti-thrombotic, anti-coagulant and anti-inflammatory effects^[45,46]. Mirroring those versatile properties, mass spectrometry analyses revealed that HDL encompasses very heterogeneous macromolecular complexes of lipids and proteins. Only one third of the up to 80 different proteins identified in HDL is dedicated to lipid transport. The remaining proteins being either acute-phase proteins, proteases, anti-oxidant, anti-thrombotic enzymes or proteins involved in complement regulation^[45,46].

In addition to being the principal protein fraction of HDL and a limiting factor for HDL formation, apoA-1 *per se* has many of the HDL-related atheroprotective properties, such as inhibition of immune cell trans-endothelial migration, inhibition of monocyte activation, inhibition of cytokine production induced by T-cell contact, inhibition of lipid peroxidation, and interference with innate immune receptors pro-inflammatory signaling^[45]. There is also a growing body of evidence indicating that both acute and chronic inflammatory conditions induce post-translational modifications of apoA-1 transforming HDL and apoA-1 into pro-inflammatory molecules^[46]. Furthermore, as reviewed in the next paragraphs, recent data suggest that that humoral autoimmunity to apoA-1 and HDL could be new possible biomarkers for CVD, and possibly a mediator of inflammation, atherosclerosis, and CVD.

ANTI-APOA-1 IGG IN AUTOIMMUNE DISEASES

Anti-apoA-1 IgG in SLE and APS patients

In 1995, using early phage display technology, Merrill *et al.*^[47] reported that sera derived from SLE patients were immunoreactive against a protein displaying 82% DNA sequence homology with human apoA-1, followed by the confirmation that those sera were indeed reactive to human apoA-1 when coated on gamma-

irradiated enzyme-linked immunosorbent assay plates. Further understanding of anti-apoA-1 autoantibody architecture was provided by the same group in 1998 who reported that high levels of anti-apoA-1 IgG were retrieved in a significant subset of SLE (32.5%) and primary APS patients (22.9%)^[35]. Those autoantibodies were found to be associated with the presence of anti-beta2glycoprotein I (β 2GPI) antibodies, and to display an optimal affinity for mature HDLs^[48]. In 2001, Abe and colleagues characterized six different monoclonal anti-apoA-1 antibodies (derived from two SLE patients) displaying a low specificity, as reflected by their broad cross-reactivity to single strand DNA, thrombin, cardiolipin (CL), and to HDL^[48,49]. Because of the latter observation, anti-apoA-1 IgG were considered a possible subgroup of anti-HDL antibodies^[49]. The first insight regarding the potential pathogenicity of this class of autoantibodies in atherogenesis was demonstrated in 2003 by Delgado Alves and colleagues, who showed an inverse correlation between anti-HDL IgG and paraoxonase-1 (PON-1) activity, and with the total antioxidant capacity of the corresponding sera^[50]. More specifically, those initial results suggested that anti-HDL, and later anti-apoA-1 IgG^[51], could be related to atherogenesis, through HDL dysfunction^[52,53], whose pathophysiological role in atherogenesis was starting to be recognized^[54].

Anti-apoA-1 IgG in rheumatoid arthritis patients

In 2010, we demonstrated in a case-control study that anti-apoA-1 IgG levels were higher in patients suffering from rheumatoid arthritis (RA) than in matched-controls (17% *vs* 2%, $P = 0.01$)^[55]. In this study, those autoantibodies were associated with higher oxidized LDL levels and were significantly associated with anamnestic CVD. Nevertheless, no association was found with the RA disease activity score^[55]. Concomitantly, in a longitudinal prospective study which will be described in detail in the following paragraph entitled “anti-apoA-1 IgG as independent predictors of CV risk“, we confirmed that those autoantibodies were predictive of CVD in RA patients, and were associated with a pro-inflammatory cytokine profile^[56,57].

ANTI-APOA-1 IGG IN OTHER NON-AUTOIMMUNE POPULATIONS

If high levels of anti-apoA-1 IgG are initially described as raised in patients with autoimmune diseases associated with an increased risk of CVD, high levels of those autoantibodies can also be detected in patients without autoimmune disease, but with CVD, such as acute coronary syndrome^[58-62], and severe carotid stenosis^[63,64].

In addition, the existence of elevated levels of anti-apoA-1 IgG was demonstrated in patients with periodontitis^[65], and patients under hemodialysis^[66], two clinical conditions known to be associated with increased CVD risk^[67,68]. Finally, the existence of high levels of anti-apoA-1 IgG was recently reported in obese, but other-

wise healthy subjects^[69].

In those different settings the prevalence of a high titer of anti-apoA-1 IgG varies between 10% and 20%, against 0% to 6.5% in healthy blood donors or controls^[56,59,65]. The clinical relevance of such findings will be discussed later.

ANTI-APOA-1 IGG AS A MARKER AND POSSIBLE MEDIATOR OF INFLAMMATION AND ATHEROGENESIS

Anti-apoA-1 IgG are associated with a pro-inflammatory and pro-atherogenic cytokine profile in humans

Most studies published to date have reported significant associations between high levels of anti-apoA-1 IgG levels and markers of oxidative stress, inflammation and endothelial dysfunction related to atherogenesis and atherosclerotic plaque rupture.

In SLE patients, anti-apoA-1 IgG levels were found to be positively correlated with nitric oxide ($r = 0.37$, $P = 0.007$), inversely related to PON-1 activity ($r = -0.31$, $P = 0.006$), and the total anti-oxidant capacity of the sera ($r = -0.47$, $P < 0.0001$) suggesting that those autoantibodies could interfere with the anti-oxidant properties of HDL, giving rise to a pro-oxidative micro-environment facilitating atherogenesis^[70]. Similarly, RA patients tested positive for those autoantibodies were shown in two different studies to have higher plasma levels of oxLDL levels^[55,56], considered a major player in all stages of atherogenesis^[2,10,11]. Furthermore, RA patients tested positive for anti-apoA-1 antibodies were found to have higher levels of interleukin-8 (IL-8) and MMP-9^[55], two inflammatory mediators known to be associated with atherogenesis, and atherosclerotic plaque vulnerability in humans^[14,71].

In a retrospective study involving MI patients, we reported a positive association between anti-apoA-1 IgG and serum amyloid A (SAA) protein levels ($r = 0.76$, $P = 0.006$), a multifunctional protein located at the crossroad of inflammation and cholesterol homeostasis^[58]. Subsequently, in a prospective cohort study involving MI patients ($n = 127$), we noted the same relationship between anti-apoA-1 IgG and oxLDL levels as had been documented in RA patients^[59]. MI patients considered as positive for anti-apoA-1 IgG had significantly higher median levels of oxLDL when compared to patients tested negative for those autoantibodies (226.5 U/L *vs* 47.7 U/L, $P < 0.0001$), and a positive correlation between oxLDL and anti-apoA-1 IgG was observed (Spearman $r = 0.28$, $P < 0.05$)^[59]. On the other hand, no association with PON-1 activity was observed in this study.

In a prospective study enrolling 221 MI patients, we demonstrated that patients tested positive for anti-apoA-1 antibodies had higher circulating levels of IL-6, TNF- α , and MMP-9, and lower MMP-3 levels^[72], a cytokine constellation known to be associated with increased atherosclerotic plaque vulnerability, and worse CV prognosis^[73,74]. This increase in MMP-9 levels retrieved in anti-apoA-1 IgG positive patients was associated with an

increase in MMP-9 activity^[63].

Furthermore, in our periodontitis study, we observed a positive correlation between anti-apoA-1 IgG and ADMA levels (Spearman; $r = 0.20$, $P = 0.02$)^[65], a marker of endothelial-dependent dysfunction with strong CV prognostic value^[75,76].

Among other associations observed between anti-apoA-1 IgG CV relevant prognostic features was an association with basal heart rate. In one of our prospective MI cohort studies^[60], we demonstrated that when compared to those tested negative for anti-apoA-1 IgG, patients tested positive for those antibodies had a higher basal heart rate upon discharge, a well-established CV prognostic feature after MI^[77-79]. The possible impact of those autoantibodies on nervous autonomic dysfunction will be presented in the paragraph entitled "Anti-apoA-1 IgG elicits a positive chronotropic effect on cardiomyocytes".

In conclusion, the most consistent associations observed so far between anti-apoA-1 IgG and CV-relevant markers of inflammation concern mostly oxLDLs and MMP-9. Although no causal link can be inferred based on such statistical associations, they were nevertheless instrumental in orienting the subsequent *in vitro* and animal studies described in the next paragraph.

Anti-apoA-1 IgG as active mediators of atherosclerosis and atherosclerotic plaque vulnerability in vitro and in vivo

Experiments carried out in cellular and animal models indicated that certain autoantibodies contribute directly to the induction of atherogenesis and atherosclerotic plaque vulnerability through their capacity to signal through innate immune receptors, notably TLR-2⁽²⁹⁻³¹⁾, reviewed in^[27]. By analogy, we investigated whether anti-apoA-1 autoantibodies could act through innate immune receptors signaling to elicit a pro-inflammatory response.

In this respect, we recently showed that lipopolysaccharide-free anti-apoA-1 IgG dose-dependently induced the production of a range pro-inflammatory cytokines, such as IL-8, MMP-9, IL-6, TNF- α , and MCP-1 in human monocyte-derived macrophages^[55,63,72], and that this process was mediated by the TLR2/CD14 complex^[72]. In addition, our *in silico* modeling studies revealed evidence of structural homology between apoA-1 and part of the extracellular domain of TLR2, suggesting a molecular mechanism for this cross-reactivity^[72]. Our current understanding on how anti-apoA-1 IgG promotes sterile inflammation through the activation of TLR2/CD14 complex is summarized in Figure 3.

Anti-apoA-1 IgG elicits a positive chronotropic effect on cardiomyocytes

We have recently demonstrated that there is a positive association between levels of anti-apoA-1 IgG and resting heart rate following myocardial infarction, a well-established parameter for CVD prognosis in secondary prevention^[60,79]. In the same study, we showed that in the presence of aldosterone, anti-apoA-1 IgG elicits a

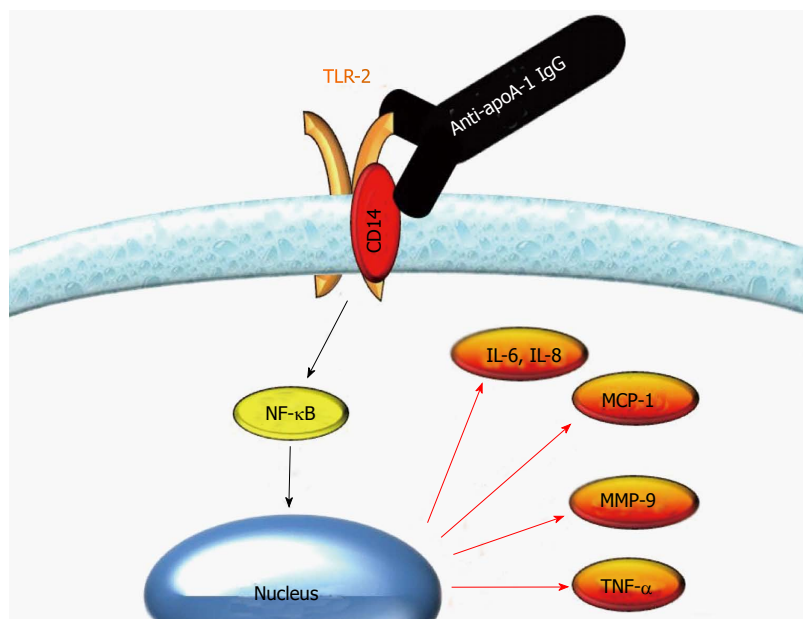


Figure 3 Autoantibodies against apolipoprotein A-1 IgG elicit a pro-inflammatory response through Toll-like receptor 2/CD14 complex on human macrophages. Autoantibodies against apolipoprotein A-1 (anti-apoA-1) IgG specifically bind to Toll-like receptor (TLR)2 due to conformational homology between apoA-1 and TLR2. In the presence of CD14, the binding of anti-apoA-1 IgG to TLR2 induces a nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-dependent production of pro-inflammatory cytokines. MMP-9: Matrix-metalloproteinases; IL-8: Interleukin-8.

dose-dependent increase in the spontaneous contraction rate of neonatal rat ventricular cardiomyocytes^[60]. Using patch-clamp electrophysiology combined with a pharmacological approach, we subsequently showed that this positive chronotropic effect was mediated by L-type calcium channel activation, itself induced by the concomitant activation of both the mineralocorticoid receptor-dependent phosphatidyl 3-kinase pathway and the protein kinase A pathway^[80]. In support of an activation mechanism involving aldosterone and antibody, we demonstrated that the chronotropic effect can be abrogated by addition of eplerenone, an aldosterone antagonist, and by intravenous immunoglobulins^[60,80]. Hence, there is compelling evidence in support of a role for anti-apoA-1 IgG in the induction of a positive chronotropic effect in cardiomyocytes, but further work will be required to define (1) whether this is a direct or indirect effect; and (2) if anti-apoA-1 IgG acts directly on cardiomyocytes, which receptor does it engage to activate the protein kinase A pathway (Figure 4)? At the present time, there is no indication suggesting that this chronotropic effect could be mediated by interference with the activity of the autonomous nervous system; further work will be required to address this question.

Anti-apoA-1 IgG induces atherosclerosis and death in apoE^{-/-} mice

Animal studies that we have performed provided direct evidence that anti-apoA-1 IgG was sufficient to induce atherosclerosis. Passive immunization of atherosclerosis-prone apoE^{-/-} mice with anti-apoA-1 IgG increased both atherosclerotic lesion size and histological features of atherosclerotic plaque vulnerability^[63]. In a lupus-prone mouse model, Srivastava and colleagues demonstrated that the presence of anti-apoA-1 antibodies was associated with a decrease in the anti-oxidant properties of HDL which inferred a decrease in PON-1 activity, leading to an increase in pro-inflammatory reactive oxygen species^[81].

These results support the hypothesis that anti-apoA-1 IgG and HDL dysfunction are two related phenomena. Although a causal link between anti-apoA-1 IgG and HDL dysfunction remains elusive, these results are consistent with clinical observations reported previously^[49-52].

ANTI-APOA-1 IGG AS INDEPENDENT PREDICTORS OF CV RISK

In 2010, we demonstrated that anti-apoA-1 IgG positivity assessed in samples taken within the first 24 h of patient admission for MI was a significant and independent predictor of MACE during 1 year follow-up^[60]. The presence of high anti-apoA-1 IgG levels on admission increased the subsequent risk of MACE by 4-fold, independently of Framingham risk factors [adjusted OR = 4.3, 95%CI: 1.46-12.6, $P = 0.007$]^[60]. Cox regression analysis demonstrated that for each arbitrary unit increase in anti-apoA-1 IgG, there was a concomitant 3% increase in MACE risk ($P = 0.0003$). All 221 patients tested negative for anti-nuclear antibodies and no association with any other autoantibodies (rheumatoid factor, anti-β2GPI and anti-cardiolipin antibodies) was observed^[60].

These findings were extended in an ancillary study derived from the same cohort of patients aimed at comparing, in a “head to head” fashion, the prognostic accuracies of other autoantibodies described as potentially relevant for CV event prediction. Among those, we measured antibodies to β2GPI domain I and IV, cardiolipin, heat-shock protein 60 (anti-HSP-60), and phosphorylcholine (anti-PC IgM)^[61]. In this study, autoantibodies to apoA-1 were found to be the only autoantibodies to significantly predict subsequent MACE occurrence, although a non-significant trend was observed for anti-cardiolipin ($P = 0.05$), and anti-HSP60 antibodies $P = 0.07$). In this study, the prognostic accuracy measured by the area under the curve (AUC) was rather modest (AUC: 0.65, $P = 0.007$)^[61], and of the same order of magnitude as the 10-year global

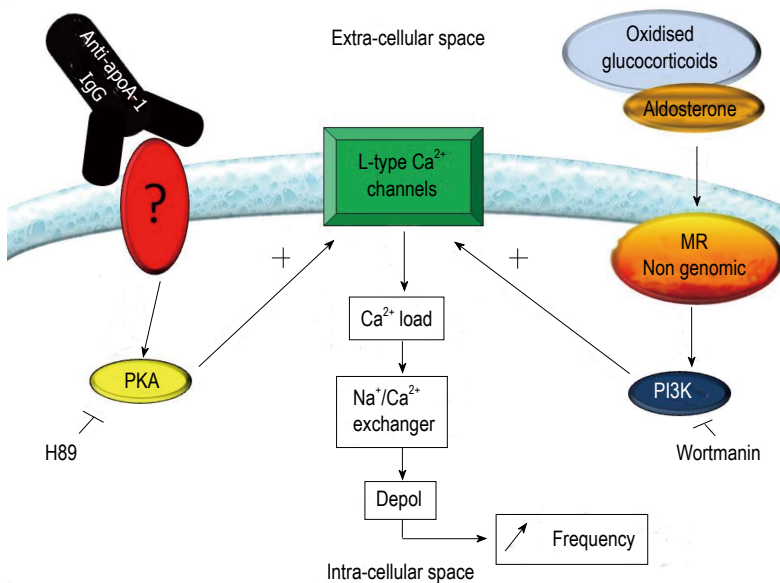


Figure 4 Current understanding of the mechanism by which autoantibodies against apolipoprotein A-1 IgG elicit chronotropic responses in neonatal rat cardiomyocytes. Stimulation of the mineralocorticoid receptor (MR), either by aldosterone or oxidized glucocorticoids, induces the downstream activation of PI3K, which in turn activates L-type calcium channels. Anti-apoA-1 IgG has been shown to sensitize the L-type calcium channel in a protein kinase A (PKA)-dependent manner. The PI3K and PKA activated pathways alone are not sufficient to induce an increase in basal contraction rate, when simultaneously activated L-type calcium channels are activated, leading to an increase in intracellular Ca^{2+} . This signal is amplified by the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, leading to an increase of the prepotential slope of the cells, which ultimately translates into an increased contraction rate.

Framingham risk score. Risk analyses demonstrated that anti-apoA-1 IgG positivity increased the risk of MACE by 4-fold, independently of the 10-year global Framingham risk score (adjusted hazard ratio = 3.8, $P = 0.002$)^[61]. Those preliminary results pointed to anti-apoA-1 IgG as a promising humoral autoimmune candidate for MACE prediction in secondary prevention settings.

Furthermore, in a single center prospective study involving 138 patients presenting to the emergency room with acute chest pain, we demonstrated that anti-apoA-1 IgG values assessed on the first sample available had a relatively good diagnostic accuracy for non-ST elevation myocardial infarction (NSTEMI) with an AUC of 0.75 ($P < 0.0001$) that could be increased up to 0.88 when combined with anti-PC IgM and the NSTEMI-TIMI score to generate a clinical antibody ratio (CABR) score^[62]. Also, anti-apoA-1 IgG was found to be a good predictor (AUC = 0.80, $P < 0.0001$) of subsequent troponin I elevation when the first sample tested negative, which was the secondary endpoint of this study. Risk analyses indicated that in the presence of high anti-apoA-1 IgG levels, the risk of subsequent NSTEMI diagnosis was increased by 6-fold after the adjustment for NSTEMI-TIMI score (OR: 6.4, 95%CI: 1.72-24.2). At the pre-specified cut-off, this test displayed an interesting negative predictive value of 88% and 95% for the primary and secondary study endpoints, respectively. To summarize, in ACS patients, the predictive accuracy according to ROC curve analysis revealed AUC values ranging between 0.65 and 0.75^[61,62]. If these AUC values are relatively modest (they should ideally be above 0.80^[36]), they are still in the same range as those reported for the Framingham risk score, which currently determines patient management^[3].

Furthermore, we demonstrated that anti-apoA-1 IgG were also predictors of MACE at one-year after elective surgery for severe carotid stenosis with an AUC of 0.74 (95%CI: 0.59-0.90, $P = 0.01$)^[64], and that its combined used with myeloperoxidase could improve the predictive accuracy of the model^[64]. In this study, high levels of

anti-apoA-1 IgG were associated with a 5-fold increase in MACE during follow-up (exact OR = 5.29, 95%CI: 1.08-34.02, $P = 0.04$), which remained significant after adjustment for the 10-year Framingham risk score according to conventional logistic regression, but not when the exact logistic regression model was applied^[64].

In a longitudinal prospective study involving 133 RA patients followed-up for a median duration of 9 years^[59], we demonstrated that high levels of anti-apoA-1 IgG was associated with a 4-fold increase in MACE during follow-up, independently of Framingham risk factors and RA disease duration (HR = 4.2, 95%CI: 1.5-12.1). In this study, ROC curve analyses indicated that those autoantibodies were the strongest predictors of MACE with an AUC of 0.73 ($P = 0.0008$), a specificity of 50%, and a sensitivity of 90% at the predefined cut-off^[59]. In addition to their independency of traditional CV risk factors to predict poor CV outcome, we also demonstrated that anti-apoA-1 IgG provides incremental prognostic information over traditional cardiovascular risk factors in ACS, in severe carotid stenosis, and in RA patients. When compared to current risk stratification tools (NSTEMI-TIMI score in acute chest pain patients, or the 10-year global Framingham risk score in ACS, RA, or severe carotid stenosis patients), it significantly improved the patient risk reclassification with significant integrated discrimination index values ranging between 1.8% and 175%^[57,62,64].

Anti-apoA-1 IgG as a biomarker predictive of atherosclerosis and atherosclerotic plaque vulnerability

Of clinical relevance, we have also demonstrated that anti-apoA-1 IgG is also detectable in a proportion of healthy subjects without autoimmune disease and CVD (0%-6.5%), albeit at lower levels than seen in patient cohorts^[56,59,65]. Significantly, in a small case-control study on healthy subjects^[69], we demonstrated that anti-apoA-1 IgG levels in the obese subgroup were raised to levels previously described in CVD patients, with high levels of anti-apoA-1 IgG being a significant predictor of coro-

nary artery calcifications visualized by chest computed tomography. Because coronary artery calcifications are a major predictor of subsequent cardiovascular events in asymptomatic subjects^[82], the results of this preliminary study suggest that anti-apoA-1 IgG may be a valuable biomarker for use in primary prevention to screen for the presence of coronary artery lesions. Indeed in this setting, anti-apoA-1 IgG testing had a negative predictive value of 94% to detect the presence of coronary artery calcification, with an AUC of 0.83^[69]. Similarly, we demonstrated in patients with periodontitis younger than 50 years old that anti-apoA-1 IgG was the only predictor of a pathological ankle brachial index^[65], a measure used to detect peripheral artery disease and known to reflect the global atherosclerosis burden^[83,84].

Extending those results, we also reported that the presence of anti-apoA-1 antibodies in patients with severe carotid stenosis was associated with histological features of atherosclerotic plaque vulnerability determined on surgical biopsy specimens^[63]. Indeed, in this study, we demonstrated that circulating levels of anti-apoA-1 IgG were positively correlated with intraplaque macrophages ($r = 0.33$, $P = 0.002$), MMP-9 expression ($r = 0.43$, $P = 0.0001$) and neutrophils ($r = 0.42$, $P = 0.0001$), and inversely correlated with total collagen content ($r = -0.29$, $P = 0.008$). Furthermore, patients deemed as positive for anti-apoA-1 IgG had significantly higher levels of macrophages, MMP-9 expression and neutrophils within their atherosclerotic lesions, and lower levels of total collagen when compared to patients tested negative for those autoantibodies^[63]. Interestingly, those findings were mimicked in apoE^{-/-} mice exposed to passive immunization with anti-apoA-1 IgG when compared to the CTL group^[63]. Taken together those results indicate that assessing anti-apoA-1 IgG levels could not only be a possible biomarker of atherosclerosis, but could also be used to detect the presence of atherosclerotic plaque vulnerability. Because assessing atherosclerotic plaque vulnerability is currently an unmet clinical need, the possibility of using anti-apoA-1 IgG detection as a simple and affordable surrogate biomarker of atherosclerotic plaque fragility is of patent clinical interest.

FUTURE PERSPECTIVES

Because current *in vitro* and *in vivo* results indicate that anti-apoA-1 IgG could well be active mediators of atherogenesis, those autoantibodies may represent an emergent therapeutic target. In other words, we speculate that measuring circulating levels of anti-apoA-1 IgG would enable the identification of a subset of patients who would benefit from specific therapy aimed at reversing the deleterious effect of those autoantibodies. In this respect, we have demonstrated that the chronotropic effect of those autoantibodies could be reversed by existing therapeutic compounds such as IVIG and eplerenone, a selective MR antagonist^[80].

In parallel, we will pursue our work aimed at defining

the exact CV-relevant epitope(s) targeted by those autoantibodies. Once determined, those epitopes could be useful both for the detection of anti-apoA-1 IgG by occupying binding sites, and for neutralizing the pathogenic effects of the antibodies (pro-arrhythmogenic and pro-inflammatory effects), which hopefully would translate in a reduction of atherogenesis-related complications in humans.

CONCLUSION

To summarize, recent studies demonstrate that IgG autoantibodies against apoA-1 are raised in many diseases associated with a high cardiovascular risk, such as SLE, ACS, RA, severe carotid stenosis, and end-stage renal disease. To date, high levels of anti-apoA-1 IgG have been shown to be an independent prognostic marker of poor CV outcome in MI, RA and carotid stenosis patients, to display clinically relevant properties for NSTEMI diagnosis in acute chest pain patients, to be associated with atherosclerotic plaque vulnerability in patients with severe carotid stenosis, and to predict coronary artery lesions in obese, but otherwise healthy subjects. In most studies reported so far, high levels of anti-apoA-1 IgG are associated with a pro-inflammatory cytokine profile, and in SLE/APS, those autoantibodies have been shown to be associated with the presence of dysfunctional HDLs.

Concomitantly, *in vitro* data tend to indicate that anti-apoA-1 IgG are active modulators of atherogenesis by (1) promoting a sterile inflammation through the TLR2/CD14 complex; and (2) eliciting specific neutrophil chemotaxis. Furthermore, *in vitro* experiments suggest that those autoantibodies could act as pro-arrhythmogenic molecules through an aldosterone-dependent L-type calcium channel activation that can be reversed using existing therapeutic compounds. In parallel, work in mouse models demonstrated that passive immunization with anti-apoA-1 IgG increases atherogenesis, atherosclerotic plaque vulnerability, death rate, and decreases the antioxidant properties of HDL by inhibiting PON-1 activity. The preliminary clinical results need to be replicated in larger multicenter cohorts and further basic science studies will be required to gain a better understanding of the pathophysiological involvement of anti-apoA-1 IgG in atherogenesis. Nevertheless, the current converging *in vitro* and animal observations lend weight to the hypothesis that anti-apoA-1 IgG are active mediators of atherogenesis rather than innocent bystanders. Hence, these autoantibodies, could in the future, represent a new possible therapeutic target, whose deleterious effect could be abrogated by therapeutic synthetic apoA-1 mimetic peptides. In this context, anti-apoA-1 IgG appears to be a promising biomarker of pathological cardiovascular autoimmunity, allowing the identification of a subset of CVD patients who could benefit from specific immunomodulation in the future, substantially contributing to the development of personalized medicine in the field of CVD.

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Elevated blood pressure: Our family's fault? The genetics of essential hypertension

Aniket Natekar, Randi L Olds, Meghann W Lau, Kathleen Min, Karra Imoto, Thomas P Slavin

Aniket Natekar, Randi L Olds, Meghann W Lau, Kathleen Min, Karra Imoto, Thomas P Slavin, The John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96813, United States

Thomas P Slavin, Hawaii Community Genetics, Honolulu, HI 96814, United States

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Correspondence to: Thomas P Slavin, MD, Assistant Professor of Pediatrics, The John A Burns School of Medicine, University of Hawaii, 1441 Kapiolani Blvd, Suite 1800, Honolulu, HI 96813, United States. thomas.slavin@kapiolani.org
Telephone: +1-808-9733403 Fax: +1-808-9733401

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Abstract

AIM: To provide an updated review on current genetic aspects possibly affecting essential hypertension (EH), and to further elucidate their role in EH.

METHODS: We searched for genetic and epigenetic factors in major studies associated with EH between Jan 2008-Oct 2013 using PubMed. We limited our search to reviews that discussed mostly human studies, and were accessible through the university online resource. We found 11 genome wide association studies (GWAS), as well as five methylation and three miRNA studies that fit our search criteria. A distinction was not made between genes with protective effects or negative effects, as this article is only meant to be a summary of genes associated with any aspect of EH.

RESULTS: We found 130 genes from the studies that met our inclusion/exclusion criteria. Of note, genes with

multiple study references include: *STK39*, *CYP17A1*, *MTHFR-NPPA*, *MTHFR-NPPB*, *ATP2B1*, *CSK*, *ZNF652*, *UMOD*, *CACNB2*, *PLEKHA7*, *SH2B3*, *TBX3-TBX5*, *ULK4*, *CSK-ULK3*, *CYP1A2*, *NT5C2*, *CYP17A1*, *PLCD3*, *SH2B3*, *ATXN2*, *CACNB2*, *PLEKHA7*, *SH2B3*, *TBX3-TBX5*, *ULK4*, and *HFE*. The following genes overlapped between the genetic studies and epigenetic studies: *WINK4* and *BDKRB2*. Several of the identified genes were found to have functions associated with EH. Many epigenetic factors were also correlated with EH. Of the epigenetic factors, there were no articles discussing siRNA and its effects on EH that met the search criteria, thus the topic was not included in this review. Among the miRNA targets found to be associated with EH, many of the genes involved were also identified in the GWAS studies.

CONCLUSION: Genetic hypertension risk algorithms could be developed in the future but may be of limited benefit due to the multi-factorial nature of EH. With emerging technologies, like next-generation sequencing, more direct causal relationships between genetic and epigenetic factors affecting EH will likely be discovered creating a tremendous potential for personalized medicine using pharmacogenomics.

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Key words: Essential hypertension; Epigenomics; Genome-wide association study; Genes; MicroRNAs

Core tip: Essential hypertension (EH) is considered a multifactorial disease, indicating that many genetic, epigenetic, and environmental influences affect the initiation and continuance of the disease. Our goal is to provide an updated report on current genetic aspects possibly affecting EH by elucidating genetic factors' role in EH. We found 130 genes meeting our inclusion/exclusion criteria. To our knowledge, this is the first review to discuss both genetic and epigenetic factors associated with EH in one article. With emerging technologies, more direct causal relationships between

genetic and epigenetic factors with EH will likely be discovered, creating tremendous potential for personalized medicine using pharmacogenomics.

Natekar A, Olds RL, Lau MW, Min K, Imoto K, Slavin TP. Elevated blood pressure: Our family's fault? The genetics of essential hypertension. *World J Cardiol* 2014; 6(5): 327-337 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i5/327.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i5.327>

INTRODUCTION

Approximately 1 in 3 American adults, or about 67 million people, have hypertension (HTN)^[1]. According to the American Heart Association, the majority of Americans who have had first heart attacks, first strokes, or chronic heart failure had underlying HTN, a known risk factor for each condition^[2]. HTN costs the United States approximately \$47.5 billion annually in direct medical costs and roughly \$3.5 billion annually in lost economic productivity^[3].

Essential hypertension (EH), the most common form of HTN^[4], is defined as an elevation in blood pressure of unknown cause and increases the risks for cerebral, cardiac, and renal complications^[5]. EH is thought to be a multifactorial disease, indicating that many factors affect the initiation and continuance of the disease^[6]. From a genetic perspective, many single nucleotide polymorphisms (SNPs), genes and epigenetic factors are associated with EH. This suggests that people with these hereditary factors might have a genetic predisposition to having high blood pressure. Additionally, since EH has idiopathic origins, environmental factors may also play an important role in the cause of the disease. Weight gain and dietary factors appear to have a major role in causing EH due to impaired renal function, though the mechanisms are not well understood^[7].

There has been some discussion on the common disease, common variant (CDCV) and common disease, rare variant (CDRV) hypotheses and their relation to complex diseases, such as EH^[8]. The CDCV hypothesis predicts that there are common disease-producing alleles/variants that are found in all human populations with a particular phenotype for a certain disease. However, insufficient data has led to scientists challenging the validity of this hypothesis and its compatibility with many diseases^[9]. Meanwhile, the CDRV hypothesis predicts that diseases with genetic predispositions may not be found commonly in the diseased human population^[10]. One study argued that with human lineage, diseases were more likely to favor multiple rare variations contributing to disease, rather than common variations contributing to disease^[11]. This is because common variations might have external factors that would have eliminated these genes from the population, while rare variants are new, contributing to disease^[12].

The purpose of this article is to provide an updated report on the current genetic aspects that could affect

EH, and to further elucidate the role of genetic factors in EH. This includes summarizing genome-wide association studies (GWAS), as well as studies that identified genes with specific physiological functions. We also summarize current knowledge of the epigenetics in EH and/or HTN.

MATERIALS AND METHODS

Since genetic factors that influence EH in the literature are broad, we looked at specific categories of genetic factors and their influence on EH. Genetic marker studies were chosen since these studies looked specifically at what genes were involved with EH, and if any had specific physiologic effects. As epigenetics has become an emerging field of interest in genetics, DNA modification related to EH is also included, specifically focusing on DNA methylation and RNA regulation studies. It is important to note that a distinction was not made between genes with protective effects or negative effects, as this article is only meant to be a summary of genes associated with any aspect of EH.

For the search criteria, specific keywords used for each category of genetic and epigenetic factors are listed below in Figure 1.

Inclusion criteria

Reviews were selected if there was a primary focus on the genes and genetic factors associated with EH. Additionally, reviews between Jan 2008-Oct 2013 were chosen to obtain the most current information. Reviews were selected that discussed human studies, with little if any focus on animal studies. Reviews were also included if there was discussion of non-European populations since EH affects many ethnicities. Lastly, the results reported from the selected reviews were limited to reviews that discussed cohorts in populations greater than 1000 individuals. Cohorts with populations > 1000 people were chosen to reduce selection bias within the primary studies, and to ensure that the genes found could apply to large populations. From the articles that were selected to be in the study, the authors identified if the genes had known pathways related to EH.

For epigenetic factors associated with EH, we included articles that discussed various epigenetic modifications and their physiologic effects, as well as specific techniques such as methylation. If the studies had relevant animal data, this was included due to the fact that there is limited epigenetic information in human studies. Articles that discussed miRNA and the association with EH were also included to ensure a more thorough gathering of data. No articles for siRNA met our search criteria. Therefore, a discussion on siRNA as it relates to EH is not provided in this article.

Exclusion criteria

Reviews were excluded if the reviews involved rare types of HTN and/or were too detailed on EH physiology. While EH physiology is important, it does not contribute to the purpose of this paper in understanding the genetic

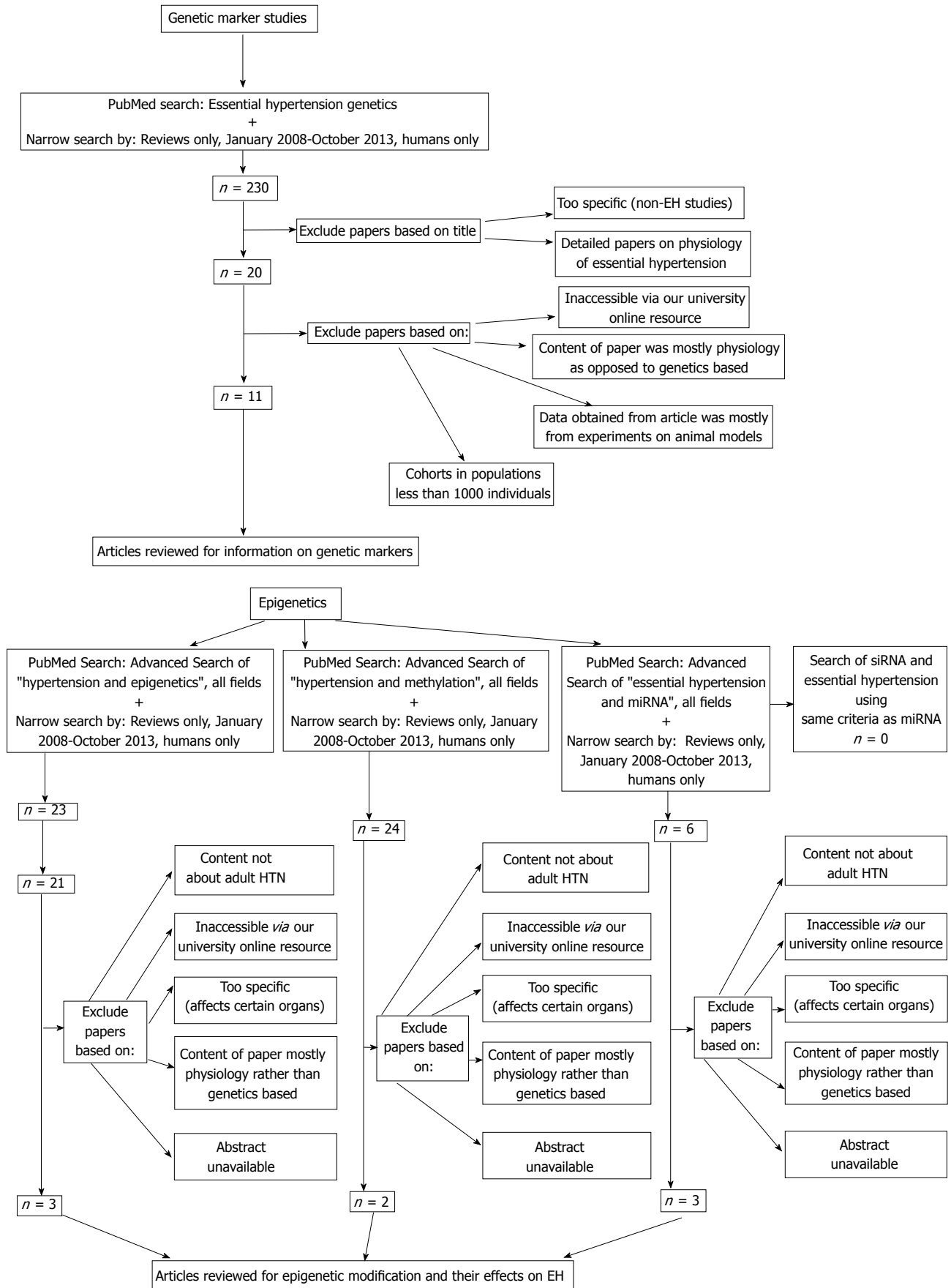


Figure 1 Search methodology for genetic and epigenetic factors associated with essential hypertension. Visual Understanding Environment v3.2.1 (Tufts University) was used to produce the images. HTN: Hypertension.

Table 1 Genetic associations with essential hypertension according to cohort

Cohort	Genes
Framingham offspring cohort	<i>CCL20-WDR69, CDH13, TGFBR2, STK39</i>
Amish cohort	<i>STK39</i>
AGEN	<i>NPR3, CYP17A1, FGF5, MTHFR, NPPA, NPPB, ATP2B1, CSK, ZNF652</i>
BP-extremes	<i>UMOD</i>
BRIGHT	<i>BCAT1</i>
CARe	<i>c21orf91, GPR98 and ARRDC3</i>
CBPgen	<i>CYP17A1, CACNB2, PLEKHA7, SH2B3, TBX3, TBX4, TBX5, ULK4</i>
CHARGE	<i>CPLX3, PLEKHA7, TBX3, UMOD, CYP17A1, CSK-ULK3, CYP1A2, NT5C2, CYP171A, PLCD3, SH2B3-ATXN2, CACNB2, SH2B3, TBX3, TBX4, TBX5, ULK4, c10orf107, BLK-GATA4, CASZ1, FGF5, MTHFR, NPPA, NPPB, ATP2B1, CSK</i>
FHS	<i>ANKMY, FOXD3</i>
GBPgen	<i>UMOD, CSK-ULK3, CYP1A2, NT5C2, CYP171A, PLCD3, SH2B3-ATXN2, ATXN2, c10orf107, GNAS-EDN3, MECOM (MDS1 locus), FGF5, MTHFR, NPPA, NPPB, ATP2B1, CSK, ZNF652</i>
GENE-centric	<i>SOX6, AGT, LSP1-TNNT3, MTHFR, NPPA, NPPB, ATP2B1, HFE</i>
Health2	<i>ATP2B1</i>
HUFS	<i>IPO7, MYLIP, PMS1, SLC24A4, YWHAZ, CACANA1H</i>
Hypergenes	<i>NOS3</i>
ICBP	<i>ADAMTS-8, ADM, BAT2-BAT5, CHIC2-PDGFRA1, EBF1, FES, FIGN, FLJ32810-TMEM133, GOSR2, GUCY1A3-GUCY1B3, JAG1, MOV10, NOV, NPR3-c5orf23, PIK3CG, PLCE1, SLC39A8, SLC4A7, NPR3, CYP17A1, CACNB2, PLEKHA7, SH2B3, TBX3-TBX5, ULK4, GNAS-EDN3, MECOM (MDS1 locus), FGF5, MTHFR, NPPA, NPPB, ATP2B1, CSK, ZNF652, HFE</i>
KARE	<i>ATP2B1</i>
KORA S3	<i>CCNG1</i>
Suita study	<i>CCBE1</i>
WGHS	<i>BLK-GATA4, CASZ1</i>
Study	<i>ADD1, ADD2, ADRB1, ADRB2, APOB, CACNA1A, CACNA1C, CLCNKB, CYBA, CYP11B2, CYP2C8, EDN1, EDNRA, GNB3, SCNN1A, SCNN1B, SCNN1G, SGK1, KCNJ1, ACE, ADRB2, AGT, APLNR, BDKRB2, CAPN13, CYP11B2, CYP19A, GNB3, MMP3</i>

Bolded genes are ones are found in multiple cohorts. The genes are identified and listed according to their respective cohorts, with a separate category to identify genes without specific references in any of the articles reviewed. Specific locations for the genes are provided where possible. Novel genes are identified, as are genes associated with physical properties.

basis for EH. Additionally, reviews were eliminated if the articles were inaccessible or the reviews focused on animal models. Genome-wide linkage studies were also excluded, as there was no consistency in the results for genetic markers associated with EH. Also, articles were excluded if their abstracts were unavailable. Lastly, articles were excluded if there was no access available through the library at the University of Hawaii medical school.

RESULTS

Genetic marker studies

A total of 11 genetic marker studies (genome-wide asso-

ciation studies) are found to contain relevant information with regards to gene associations with EH. Many of the studies identify genes within cohorts, and there are some genes identified in multiple cohorts. These can be found from references^[12-21], identified in Table 1. Furthermore, some of the genes have specific phenotypic effects, or associate with other genes and/or proteins related to EH. Some of the genes found have no known function, or the authors do not list the function. These can be found in references^[12-21], identified in Table 2. Genes listed with hyphens include all of the genes found inclusive of, and between, the genetic range listed.

Table 1 demonstrates the numerous amount of genes found to affect populations greater than 1000 individuals. There are several cohorts identified, each with multiple genes that are associated with EH. Also, there are some genes that are repeated in different cohorts, indicating that different populations have some genes in common with respect to EH.

Tables 1 and 2 contain the meta-analysis of two large studies with European subjects, Cohorts for Heart and Aging Research in Genetic Epidemiology Consortium and GlobalBPGen^[12], which reveal fourteen loci that reached genome-wide significance. These are thought to account for 1.5% of the observed variance in blood pressure^[12]. Many of the related genes have now been matched to physiologic functions (see “Known Pathway”, rows 1-6) that play a role in blood pressure (BP) regulation. Further studies were done on subjects of non-European descent, including African American, Japanese, Korean, and Han Chinese populations, which are listed as “Non-European Genes”. Table 2 specifically identifies the genes with known pathways related to EH regulation. Table 2 lists genes without a current known pathway to explain their influence on EH regulation.

Epigenetics and EH

Tables 3 and 4 identify many correlations between DNA and histone modifications, as well as miRNA-gene interactions and their effect on EH. Many of the genes identified were also identified through GWAS, indicating a possible mechanism for how the identified genes affect EH. It is important to note that the authors found no articles that discussed siRNA and its association with EH after conducting the literature search, thus the epigenetic section does not include siRNA.

DISCUSSION

To our knowledge, this is the first review to discuss both genetic and epigenetic factors associated with EH in one article. As one can see, many genetic factors are involved with EH. There are many genes from genetic marker studies that are found to have some association with EH, as seen in Table 2. Some genes do have known physiologic pathway associated with EH, however, many do not. Our literature review herein denotes 129 genes. Of note, genes/gene regions with multiple study references

Table 2 Genes with their identified physiological pathway and genes identified with their associated physiological functions related to essential hypertension

Genes	Pathway related to EH
NOS3	RAAS pathway ^[22]
SH2B3	Endothelial cell function ^[17]
AGT	Renal electrolyte balance ^[17]
NPPA	Control of extracellular fluid volume and electrolyte homeostasis ^[23]
NPPB	Involved in vasorelaxation and inhibition of renin and aldosterone ^[24]
NPR3	Involved with regulating blood volume and pressure, pulmonary hypertension, and cardiac function ^[25]
UMOD	Constitutive inhibitor of calcium crystallization in renal fluids ^[26]
CYP17A1	Involved with steroid/aldosterone synthesis. Enzyme dysfunction leads to increased levels of mineralocorticoid activating hormones ^[17]
ATP2B1	Codes for enzymes that have a critical role in intracellular calcium homeostasis ^[27]
CACNB2	Encodes for a subunit of a voltage-dependent calcium channel protein that is a member of the voltage-gated calcium channel superfamily ^[28]
SLC24A4	Encodes for a member of the potassium-dependent sodium/calcium exchanger protein family ^[29]
YWHAZ	Protein interacts with insulin receptor substrate 1 protein, suggesting a role in regulating insulin sensitivity ^[30]
ADAMTS-8	Enzyme encoded by the gene disrupts angiogenesis <i>in vivo</i> ^[31]
ADM	Protein encoded by gene may function as a hormone in circulation control ^[32]
c5 site between SUB1 and NPR3	SNP associated with SBP NPR3 encodes natriuretic peptide receptor C/guanylate cyclase C for natriuretic peptide clearance ^[33-35] Also found relationship with DBP
CACANA1H	Codes for $\alpha 1$ subunit of voltage-dependent calcium channel for heart contractions and associated with SBP in African Americans ^[36]
ENPEP	Facilitates production of angiotensin II in RAAS pathway and associated with SBP and DBP ^[33]
ADD1 and ACE	ADD1 codes for α -adducin protein that interacts with sodium channel of Na/K co-transporter and Na/K ATPase ^[37] Angiotensin converting enzyme produces angiotensin-converting enzyme which converts angiotensin I to angiotensin II in RAAS pathway ^[38]
ADD2	β -adducin is a cytoskeletal actin-binding protein implicated in glomerular lesions ^[39]
CYP11B2	Contributes to aldosterone synthesis in RAAS pathway ^[40]
AGT	Encodes angiotensinogen in RAAS pathway ^[41]
LOC344371 and RASGRP3	Activation decreases vascular responsiveness to endothelin-1 and angiotensin II in rats ^[41]
EDN3	Endothelin-3 involved in vasoconstriction ^[42]
BCAT1	Associated with salt sensitivity ^[43]
CASZ1	Zinc-finger transcription factor that is associated with DBP ^[33]
ADRB2	Ion channel involved with regulation of vasoconstriction ^[12]
CYP11B2	Enzymatic defects results in decreased aldosterone and increased salt-wasting ^[12,17]
MMP3	Gene variants affect arterial stiffness and endothelial function ^[44]
NR3C2	Involved with aldosterone signaling ^[12]
SCNN1B	C terminus deletion leads to reduced ENaC clearance and increased ENaC activity ^[12]
APLNR	Mediator of cardiovascular disease ^[45]
BDKRB2	Involved in catecholamine synthesis ^[46]
MTHFS	Involved with catecholamine binding ^[47]
SOX6	Required in transcription for maintenance of cardiac and skeletal muscle cells ^[17]

CACNA1A	Involved with regulating SBP ^[48]
CCNG1	Involved with regulation of SBP and DBP and is component of regulating hypertension ^[15]
CPLX3	Involved with regulating DBP ^[15]
CSK	Cytoplasmic tyrosine kinase involved with angiotensin II -dependent vascular smooth muscle cell contraction ^[17]
CACNA1C	Regulates calcium influx after depolarization ^[49]
CLCNKB	Involved in renal salt absorption ^[50]
EDN1	Endothelin-1 involved in vasoconstriction ^[51]
EDNRA	Endothelin receptor type A involved in vasoconstriction ^[52]
KCNJ1	Potassium channel involved with potassium homeostasis ^[53]
SCNN1A	Involved with renal sodium regulation ^[54]
SCNN1B	Involved with renal sodium regulation ^[55]
SCNN1G	Involved with renal sodium regulation ^[56]
SGK1	Activation of certain potassium, sodium and chloride channels, playing a role in cellular stress response ^[57]
SLC12A1	Cotransporter involved in sodium and chloride reabsorption in the distal convoluted tubule ^[58]
SLC12A3	Cotransporter involved in sodium and chloride reabsorption in the loop of Henle ^[59]
TNNT3	Involved in calcium-induced muscle contraction ^[60]
WNK1	Kinase involved with sodium and chloride transport ^[61]
WNK4	Kinase regulates balance between sodium chloride and potassium reabsorption in kidneys ^[62]
GOSR2	Interacts with target-localized SNAREs, allowing angiotensinogen to move between Golgi compartments, possibly leading to vasoconstriction ^[63]
GUCY1B3	Receptor for nitric oxide involved with vasodilation ^[64]
ATXN2	Possible association with regulation of GFR ^[65]
SLC4A7	Possible transporter of sodium and bicarbonate ions ^[66]
CDH13	Regulates endothelial cell growth ^[67]
Identifier information	Gene
Non-European genes	NPR3, IPO7, MYLIP, PMS1, SLC24A4, TBX3, YWHAZ, FIGN-GRB14, ALDH2, c5 site between SUB1 and NPR3, CACANA1H, SNP upstream of CCB1, ENPEP, ST7L-CAPZA1
Gene-gene interaction	ADD1 and ACE, ADD1 and ADD2, ADD1 and CYP11B2, AGT and ACE, c20q12, IMPG1, LOC344371 and RASGRP3, PCDH15, NPR3-c5orf23, CSK-ULK3, BAT2-BAT5, BLK-GATA4, GNAS-EDN3
Gene-environment interaction	Body Mass Index: ADD1, ADRB2, CAPN13, CYP11B2, CYP19A1, MMP3 Black, Male: AGT Level of physical activity: GNB3, NR3C2, SCNN1B, APLNR, BDKRB2 Oral contraceptive use: COL25A1 Preterm birth: MTHFS
Unknown function/ function could not be determined	GNAS-EDN3, NPR3-c5orf23, BLK-GATA4, ST7L-CAPZA1, CSK-ULK3, FIGN-GRB14, c10orf107, c21orf91, LSP1-TNNT3, GNAS-EDN3, BAT2, IPO7, MYLIP, PMS1, TBX3, TBX4, TBX5, ANKMY, BAT2, BAT3, BAT4, BAT5, ALDH2, SNP upstream of CCB1, BCAT1, PCDH15, c20q12, IMPG1, CAPN13, CYP19A1, GNB3, COL25A1, PCDH15, IMPG1, c5 site between SUB1 and NPR3, CHIC2-PDGRA1, APOB, HFE, CYPBA, CYP1A2, CYP2C8, EBF1, FES, FGF5, FIGN, FLJ32810, GNB3, LSP1, NOS3, TMEM133, FOXD3, GPR98, ARRD3, GUCY1A3, JAG1, MECOM (MD1 locus), MOV10, NOV, NPR3-c5orf23, NT5C2-CYP171A, PIK3CG, PLCD3, PLCE1, PLEKHA7, RPL6-PTPN11-ALDH2, SLC39A8, ULK4, ZNF652, CCL20, WDR69, TGFB2, STK39

Only genes with pathways related to EH were identified. Genes identified with their associated physiological functions associated with EH. If there were genes that coded for proteins, but these proteins were not found to affect EH, then it was listed as unknown function or the function could not be determined. Genes with hyphens indicate genome wide association studies associated genomic regions, in which the genetic pathway could not be determined and properly evaluated for its involvement with EH. EH: Essential hypertension; RAAS: Renin-angiotensin-aldosterone system; SBP: Systolic blood pressure; DBP: Diastolic blood pressure.

Table 3 DNA methylation and histone modification associated with essential hypertension

Ref.	Study	Subjects	Results	Site of modification and type
Smolarek <i>et al</i> ^[68]		Humans	5-mC significantly higher in healthy subjects than entire group of patients with EH	N/A
Wang <i>et al</i> ^[69]		Humans	Increased methylation levels observed at 2-CpG sites in comparison with normotensive controls	<i>SULF1</i> : Methylation
Liang <i>et al</i> ^[70]		Humans	Regulation of renal sodium reabsorption β-2 adrenergic stimulation → inhibition of histone deacetylase-8 in kidney → increased histone acetylation and decreased genetic transcription of <i>WNK4</i> caused increased blood pressure 11β-hydroxysteroid dehydrogenase type 2-converts active glucocorticoids to inactive glucocorticoids Promoter methylation of <i>HSD11B2</i> gene decreased expression of renal 11β-hydroxysteroid dehydrogenase type 2 affects regulation of volume and BP homeostasis ENaCα-epithelial sodium channel-affects Na ⁺ reabsorption in the distal nephron Proposed mechanism: Methylation of Lys79 of histone H3 suppresses ENaCα transcription ACE1-Angiotensin-converting enzyme ACE1-up-regulated in association with increased binding of histone 3 acetylation (H3Ac) and 4th lysine trimethylation (H3K4me3) and in association with decreased binding of histone ninth lysine residue demethylation (H3K9me2)	<i>WNK4</i> : Decreased transcription and increased histone acetylation <i>HSD11B2</i> : Promoter methylation ENaCα: Methylation of Lys79 of histone H3 <i>H3K4me3</i> : Histone 3 acetylation and 4th lysine trimethylation. <i>H3K9me2</i> : Decreased binding of histone 9 th lysine residue demethylation
Udali <i>et al</i> ^[71]	Friso <i>et al</i> ^[72]	Humans	11β-hydroxysteroid dehydrogenase 2 methylation at <i>HSD11B2</i> promoter in DNA of PBMCs of hypertensive patients inversely related to enzyme function Promoter methylation of <i>HSD11B2</i> gene plays a role in HTN	<i>HSD11B2</i> : Methylation in promoter region
	Lee <i>et al</i> ^[73]	Rats	Na ⁺ -K ⁺ -2 Cl ⁻ cotransporter 1 (NKCC1) Methylation status of NKCC1 promoter-elevated in hearts of spontaneously hypertensive rats SHRs-significant hypomethylation of NKCC1 associated with increase in gene expression contributing to HTN	<i>NKCC1</i> : Methylation in promoter region <i>NKCC1</i> : Hypomethylation in promoter region
	Riviere <i>et al</i> ^[74]	Human endothelial cell lines and rats <i>in vivo</i>	Somatic angiotensin-converting enzyme (= ACE1) Promoter methylation levels: Higher levels of methylation associated with transcriptional repression Therefore hypomethylation of promoter region of sACE could contribute to HTN	sACE: Methylation in promoter region
Millis ^[75]		Human	Methyl CpG binding protein-2 (MECP-2) Methylates and thereby silences the expression of the norepinephrine transporter gene Phenyl-ethanolamine N-methyltransferase (PMNT)-converts Norepinephrine into Epinephrine Also mimics gene-silencing actions of MECP-2 Leads to increased synaptic levels of catecholamines (increased Epinephrine release and decreased Norepinephrine reuptake) CTGF Lysine methyltransferase that methylates the histone H3K79 site of nucleosomes that inhibits the expression of CTGF (in the cells of the collecting ducts)	<i>MECP-2</i> : Methylation <i>PMNT</i> : Methylation <i>H3K79</i> : Methylation of histone site of nucleosomes

As demonstrated in Table 3, many of the genes identified undergo methylation. If the reviews discuss results from individual studies, then the separate studies are placed in the second column. The results are listed based on the gene/site of modification, along with a description of what occurs as a result of the modification. The last column provides a summary of the gene/site of modification and the type of modification that occurs at that particular site. CTGF: Connective tissue growth factor.

include: *STK39*, *CYP17A1*, *MTHFR-NPPA*, *MTHFR-NPPB*, *ATP2B1*, *CSK*, *ZNF652*, *UMOD*, *CACNB2*, *PLEKHA7*, *SH2B3*, *TBX3-TBX5*, *ULK4*, *CSK-ULK3*, *CYP1A2*, *NT5C2-CYP17A1*, *PLCD3*, *SH2B3-ATXN2*, *CACNB2*, *PLEKHA7*, *SH2B3*, *TBX3-TBX5*, *ULK4* and *HFE*. The following genes overlap between the genetic studies and epigenetic studies: *WNK4* and *BDKRB2*. While *WNK4* and *BDKRB2* are found in both genetic and epigenetic studies, it appears that *WNK4* (kinase

regulates balance between sodium chloride and potassium reabsorption in kidneys), and *BDKRB2* (involved in catecholamine synthesis) may be associated with EH through interactions with miRNA.

Prior to GWAS, studies were somewhat successful in isolating genes associated with rare monogenic forms of hypertension that are inherited in a classic Mendelian fashion. The introduction of GWAS has made it possible to identify novel loci that could not be predicted physi-

Table 4 MiRNA targets associated with essential hypertension

Ref.	Subjects	Results	miRNA targets
Xu <i>et al</i> ^[76]	Human plasma	hcmv-miR-UL112; miR-605; miR-623; let-7e; miR-516b; miR-600; kshv-miR-K12-6-3p; miR-602; miR-1252; miR-296-5p; miR-133b; miR-30 d; miR-625*; miR-1236; miR-518b; miR-1227; miR-664; miR-615-5p; miR-18b*; miR-1249; miR-324-3p; ebv-miRBART17-3p; miR-634; ebv-miR-miRBART19-5p; miR-486-5p; kshv-miR-K12-10a; kshv-miR-K12-10b	INF-1 is direct target of hcmv-miR-UL112 Indicates link between CMV infection and EH
Batkai <i>et al</i> ^[77]	Human Endothelial miRNA	miR-126 miR-217 miR-122 miR-21 miR-24 miR-27b, -130a, -210, -378, -17-92, let-7f miR-15, -16, -20a, -20b, -24, -221, -222 Renal miRNA miR-29b miR-200a, miR-200b, miR-141, miR-429, miR-205, miR-192 miRNA targeting RAAS miR-155 miR-526b and -578 miR-34a, and -34c miR-765 miR-383 miR-9 miR-124 and miR-135a miRNA targeting smooth muscle cells miR-143 and miR-145 miR-21 miR-21, -26b, -98, and -1826 miR-221 and -222 miRNA in other etiologic factors miR-296-5p, let-7e, hcmv-miR-UL112 hcmv-miR-UL1 miR-637	SPRED-1; PIK3 regulatory subunit-2; VCAM-1; CXCL12; RhoB SirT1 SLC7A1 Nitric oxide pathway Hypoxia-induced mechanism Pro-angiogenic Anti-angiogenic Fibrotic pathway; collagen genes; <i>Mmp2</i> ; <i>Itgb1</i> Biomarkers of nephrosclerosis <i>AGTR1</i> <i>AVPR1A</i> <i>BDKRB2</i> <i>TBXA2R</i> <i>NR3C2</i> <i>NEATc3</i> <i>NR3C2</i> Actin stress fibers; ACE; KLF5; myocardin; MRTF-B; calmodulin kinase II- δ PTEN; Bcl-2; cGMP signaling Nitric oxide and ANP pathway p27(Kip1), p57(Kip2) and/or c-kit Association with hypertension IRF-1 <i>ATP6V0A1</i> , chromaffin granule function Suppress expression of <i>AGTR1</i>
Fung <i>et al</i> ^[78]	Human	miR-155	Suppress expression of <i>AGTR1</i>

Table 4 demonstrates how miRNAs affect different aspects of blood pressure regulation. Also, there appears to be a link between cytomegalovirus (CMV) infections and essential hypertension; miRNA has been identified

as a possible mediator of this connection. The asterisk identified for some miRNAs^[78] are not defined in the original article, but are assumed to be a part of the proper notation for that miRNA. EH: Essential hypertension.

ologically, using non-family cohorts.

This review shows that no Mendelian variants or epigenetic factors are consistently associated with EH in the large cohort studies examined. Furthermore, it was not possible for the authors to correlate the epigenetic factors associated with the pathways identified, as there were no clear relationships between EH and the individual genes. Therefore, it can be inferred that EH follows multifactorial inheritance and insinuates that it follows the CDRV genetic hypothesis. In regards to identifying rare variants, GWAS is used for polymorphism detection, and is not set up to identify SNPs with low mean allele frequencies (MAFs) (low MAFs are usually under 1%, and sometimes even as high as 5%). Therefore, other techniques will need to be used to identify rare variants. Next-generation sequencing has revolutionized our ability to sequence thousands of genes at one time in a cost-effective manner. Using full exome or full genome sequencing of EH cohorts, next-generation sequencing will help to identify rare, as well as low-MAF, variants associated with regulating blood pressure^[12]. This will likely show the exact genetic factors responsible for EH instead of mere associations which have been the mainstay of our genetic search using GWAS. Similar high throughput techniques will likely also improve our identification of epigenetic regulators.

Insufficient evidence was found in this study to pursue single site genetic marker or epigenetic testing to provide a simple genetic risk assessment for EH. Genetic algorithms comprised of information from multiple genes and epigenetic factors, along with family history and environmental variables, could potentially be developed to provide a genetic risk assessment for EH. However, it will be difficult to know what to do with this data, since preventative factors such as exercise and a healthy diet would be recommended to anyone at any level of personal and/or family history risk for EH. A similar concept was examined in a recent publication evaluating genetic testing and type 2 diabetes^[79]. The evaluation of genomic applications in practice and prevention (EGAPP) consortium recommend against using genetic diabetic markers for risk assessments since it would be of limited benefit^[79]. Additionally, for cardiovascular morbidity, current non-genetic algorithms already exist^[80,81] that assess the risk of heart disease using a patient's medical profile.

Although risk assessments may be difficult, pharmacogenomic utility may be found by studying risk alleles in individuals and treating their HTN in a personalized manner based on the pathway affected to obtain optimal blood pressure control^[13].

To our knowledge, this is the first review to discuss both genetic and epigenetic factors associated with EH in one article. Insufficient evidence was found in this review to pursue any one single genetic test to provide a genetic risk assessment for EH.

In conclusion, while there exist genetic and epigenetic associations that play a role in EH, there are still no well-established cause-and-effect relationships for the development of EH. With emerging technologies, such as next-generation sequencing, a more direct relationship may be established between genetic and epigenetic factors and EH. Extensive algorithms for EH will likely need to be developed to incorporate these genetic risk factors, in concert with a patient's personal risk factors. However, the utility of this approach will need to be proven. There is a large potential for personalized medicine through pharmacogenomics that will come from our better understanding of the genetic factors and pathways involved in EH.

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COMMENTS

Background

Essential hypertension (EH) is thought to be a multifactorial disease, meaning that environmental and genetic factors affect the initiation and continuation of the disease. While there have been several publications discussing the genetic factors involved with EH, to date there has been no single publication that has discussed both genetic and epigenetic factors in one article.

Research frontiers

While EH is thought to be a multifactorial disease, several genetic factors have been associated with EH. In the area of genetic and epigenetic factors associated with EH, their remains a need to review the most updated information regarding genetic and epigenetic factors and discuss both in one article.

Innovations and breakthroughs

Previously, scientists would have to refer to Genome-wide association studies and epigenetic studies to understand how genetic and epigenetic factors are associated with EH. This is the first review article to discuss both genetic and epigenetic factors in one article. Also, this article discusses the most current up-to-date literature, providing a more recent understanding of genetic factors associated with EH.

Applications

Next-generation sequencing will allow scientists to analyze thousands of genes in a cost-effective manner. Using full exome or full genome sequencing of EH cohorts, next-generation sequencing will help to identify rare, as well as, low-mean allele frequency variants associated with regulating blood pressure. This will be useful in the growing field of pharmacogenomics, where medical regimens are being tailored to individuals based on specific genetic polymorphisms. This will help to personalize treatment regimens and improve the care given to patients with EH.

Terminology

Essential hypertension is a form of hypertension that has no known cause, but is responsible for most cases of hypertension. Genome-wide association studies look at the whole genome of populations of individuals who suffer from a specific condition to see if these individuals have any genes that differ from the general population without the condition in question. Pharmacogenomics is an emerging field where scientists and doctors use someone's genetic code to determine appropriate doses for medications to ensure fewer side effects and the best possible therapy.

Peer review

The present study appears well conducted for design and contents. Inclusion criteria and exclusion criteria are reasonable.

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Heart and lung, a dangerous liaison-Tako-tsubo cardiomyopathy and respiratory diseases: A systematic review

Roberto Manfredini, Fabio Fabbian, Alfredo De Giorgi, Marco Pala, Alessandra Mallozzi Menegatti, Claudia Parisi, Elisa Misurati, Ruana Tiseo, Massimo Gallerani, Raffaella Salmi, Eduardo Bossone

Roberto Manfredini, Fabio Fabbian, Alfredo De Giorgi, Marco Pala, Alessandra Mallozzi Menegatti, Claudia Parisi, Elisa Misurati, Ruana Tiseo, Clinica Medica, Azienda Ospedaliera-Universitaria of Ferrara, 44124 Cona, Ferrara, Italy
Massimo Gallerani, 1st Internal Medicine Unit, Azienda Ospedaliera-Universitaria of Ferrara, 44124 Cona, Ferrara, Italy
Raffaella Salmi, 2nd Internal Medicine Unit, Azienda Ospedaliera-Universitaria of Ferrara, 44124 Cona, Ferrara, Italy
Eduardo Bossone, Cardiology Unit, Azienda Ospedaliera-Universitaria of Salerno, 84131 Salerno, Italy

Author contributions: Manfredini R and Bossone E equally contributed to the work; Manfredini R, Fabbian F, Gallerani M, Salmi R, Bossone E designed the research; De Giorgi A, Pala M, Mallozzi Menegatti A, Parisi C, Misurati E, Tiseo R searched the available literature, found the articles, and performed the research; Manfredini R, Fabbian F, De Giorgi A, Pala M, Mallozzi Menegatti A, Parisi C, Misurati E, Tiseo R analyzed the data; Manfredini R, Fabbian F, Bossone E, Gallerani M wrote the paper; Manfredini R, Fabbian F, De Giorgi A, Pala M, Mallozzi Menegatti A, Parisi C, Misurati E, Tiseo R, Gallerani M, Salmi R, Bossone E revised critically the manuscript for intellectual and conceptual content, and approved the final version; Manfredini R collected funds.

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Correspondence to: Roberto Manfredini, MD, Clinica Medica, Azienda Ospedaliera-Universitaria of Ferrara, Via Aldo Moro 8, 44124 Cona, Ferrara, Italy. roberto.manfredini@unife.it
Telephone: +39-0532-237166 Fax: +39-0532-236816

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Abstract

AIM: To investigate the possible association between Tako-tsubo cardiomyopathy (TTC)-a reversible clinical condition mimicking an acute myocardial infarction characterized by multifactorial pathophysiologic mecha-

nisms- and respiratory system diseases.

METHODS: We systematically searched PubMed and EMBASE medical information sources, to identify the different triggering causes, limiting our search to articles in English. The search keywords were: "tako-tsubo cardiomyopathy", "takotsubo", "takotsubo cardiomyopathy", "broken heart syndrome", "stress-induced cardiomyopathy", "apical ballooning syndrome", and "apical cardiomyopathy in combination with respiratory diseases, lung, pulmonary disease. For each kind of disease, we registered: author, year and country of study, patient sex, age, concurring situation, and outcome.

RESULTS: Out of a total of 1725 articles found, we selected 37 papers reporting a total of 38 patients. As expected, most patients were women (81.6%), mean age was 65 ± 10 years. Outcome was favorable in 100% of cases, and all the patients have been discharged uneventfully in a few days.

CONCLUSION: An association between respiratory diseases and TTC is likely to exist. Patients with severe respiratory diseases, due to the high dosages of β_2 -agonists used or to the need of invasive procedures, are highly exposed to the risk of developing TTC.

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Key words: Tako-tsubo cardiomyopathy; Stress cardiomyopathy; Respiratory diseases; Lung; Chronic obstructive pulmonary disease; Asthma

Core tip: This is the first study evaluating the association between respiratory diseases and Tako-Tsubo cardiomyopathy (TTC). Patients with severe respiratory diseases, due to the high dosages of β_2 -agonists used or to the need of invasive procedures, are highly ex-

posed to the risk of developing TTC. Thus, in these patients a certain caution should be maintained, along with a special alertness in suspecting and recognizing this particular disease.

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INTRODUCTION

Tako-tsubo cardiomyopathy (broken heart syndrome)

Tako-Tsubo cardiomyopathy (TTC) is a reversible clinical condition mimicking an acute myocardial infarction (AMI)^[1]. The original Japanese term “tako-tsubo” indicates the particular shape of the end-systolic left ventricle in ventriculography resembling that of the round-bottom and narrow-neck pot used for trapping octopuses^[2]. Other terms have been used to define this cardiac entity, *i.e.*, “apical ballooning”, “acute stress cardiomyopathy” or “broken heart”. Typical presentation involves chest pain and/or dyspnea, transient ST-segment elevation on the electrocardiogram (ECG), and a modest increase in cardiac troponin^[3].

The Mayo clinic diagnostic criteria include: (1) transient hypokinesia, akinesia or dyskinesia in the left ventricular mid segments with or without apical involvement; regional wall motion abnormalities that extend beyond a single epicardial vascular distribution; and, frequently but not always, a stressful trigger; (2) absence of obstructive coronary disease or angiographic evidence of acute plaque rupture; (3) new ECG abnormalities (ST-segment elevation and/or T-wave inversion) or modest elevation in cardiac troponin and, and (4) absence of myocarditis or pheochromocytoma^[1].

Although TTC is still underdiagnosed, the current prevalence estimate is approximately 1% to 3% (even 6% to 9% in women) of all acute coronary syndromes^[4]. The mean age ranges from around 60 to 75 years, both in men and women^[5], but its occurrence is much more likely (approximately 90%) in postmenopausal women^[6]. After a first finding on a large cohort of patients in Italy^[7], a precise temporal periodicity has been reported, characterized by highest occurrence peaks during morning hours and summer months^[8-9]. Interestingly, quite similar to AMI, Monday seems to be a critical day for onset^[10].

Even if TTC is frequently characterized by dramatic clinical presentation and urgent presentation to the Emergency Department, the prognosis is generally favorable, with a rapid short-term improvement of left ventricle systolic function^[11]. According to several studies, in-hospital mortality rates range from 0% to 8%^[4], with higher mortality rates for males than females^[12].

Multifactorial pathophysiologic mechanisms are likely to be involved, but the most accepted pathogenic hypothesis considers a rapid elevation of circulating catecholamine, triggered by emotional and/or physical stress, as a key mechanism^[13-14]. In fact, the major determinants of sympathetically mediated myocardial reversible dysfunction in patients with TTC include all the direct effects of catecholamines upon the myocardium, *i.e.* cellular damage, contraction band necrosis, defects in perfusion, altered cellular metabolism, and negative inotropic effects of epinephrine *via* stimulation of the cardioprotective β_2 -adrenergic receptors-Gi signaling pathway^[15]. It has been recently shown that the apical ventricular region has a greater $\beta_2:\beta_1$ adrenoceptor ratio, with a higher responsiveness and vulnerability to sympathetic stimulation^[16]. Again, the different occurrence of wall motion abnormalities could be explained by interindividual anatomical differences in the distribution of β -adrenergic receptors^[17,18].

Broken heart and broken lung: Is there a relationship?

The clinical onset of TTC is usually preceded by an emotional and/or physical stress with a similar distribution in approximately two-thirds of the patients. A long list of stressors has been reported, and this is continuously updated. Men seem to be more prone to physical stress and women to emotional stress^[19]. Among emotional stressors, for example, death or severe illness of a family member, receiving bad news, financial loss, move to a new residence, natural disasters, dispute or litigation, car accident, assault, surprise party, public speaking, and so on^[3]. Among physical stressors, surgery, cardiovascular procedures, medications and illicit drugs, and medical conditions, including gastroenterologic, endocrine, hematologic, renal, infectious, and neurologic diseases^[3]. Thus, we aimed to more-in-depth investigate the relationships between TTC and respiratory diseases.

MATERIALS AND METHODS

We systematically searched PubMed and EMBASE medical information sources, to identify the different triggering causes, limiting our search to articles in English. The search keywords were: “TTC”, “takotsubo”, “takotsubo cardiomyopathy”, “broken heart syndrome”, “stress-induced cardiomyopathy”, “apical ballooning syndrome” and “ampulla cardiomyopathy in combination with respiratory diseases, lung, pulmonary disease. Further papers were sought by means of manual search of secondary sources, including references from primary articles. For each kind of disease, we collected a set of data, including author, year of publication, country where the study was performed, and patient sex, age, concurring situation, and outcome.

RESULTS

Out of a total of 1725 articles found (1341 with the precise MeSH term (Takotsubo cardiomyopathy), we se-

Table 1 Respiratory symptoms or diseases and Tako-tsubo cardiomyopathy: Synopsis of published case reports

Symptom/disease	Gender, age (yr)	Concurring condition	TTC outcome	Country	Ref.
COPD					
Dyspnea	Female, 57	COPD	Favorable	United States	Pezzo <i>et al</i> ^[26]
Dyspnea	Female, 51	Unexpected death of her son COPD, Hypothyroidism Financial problems	Favorable	Poland	Bilan <i>et al</i> ^[27]
Status asthmaticus	Female, 66	COPD with multiple hospitalizations, heavy smoker	Favorable	United States	Rennyson <i>et al</i> ^[28]
Exacerbation	Female, 63	Multiple admissions	Favorable	United States	Makam <i>et al</i> ^[24]
Exacerbation	Male, 52	Financial unavailability to buy his drugs	Favorable	Spain	Pham <i>et al</i> ^[29]
Exacerbation	Female, 62	(1) COPD exacerbation (2) Family dispute (3) Acute thrombosis of aortobifemoral prosthesis	Favorable	Germany	Sager <i>et al</i> ^[30]
Exacerbation	Female, 68	COPD β2 agonist abuse	Favorable	Brazil	Salemi <i>et al</i> ^[21]
Exacerbation	Female, 63	Severe longstanding COPD, heavy smoker	Favorable	New Zealand	White <i>et al</i> ^[22]
	Male, 59	Ex-smoker, COPD Salbutamol abuse			
Exacerbation	Female, 76	COPD	Favorable	United States	Mendoza <i>et al</i> ^[23]
Exacerbation	Female, 63	β2 agonist abuse multiple exacerbations with noninvasive ventilation	Favorable	United States	Laktikova <i>et al</i> ^[25]
Asthma					
Bronchial asthma	Female, 74	Jet lag, 3 cups of coffee, 1-h sauna	Favorable	Taiwan	Chang <i>et al</i> ^[37]
Allergic rhinitis	Female, 84	Nasal decongestant abuse	Favorable	Brazil	Wang <i>et al</i> ^[34]
Status asthmaticus	Female, 46	Ketamine + epinephrine administration	Favorable	United States	Osuorji <i>et al</i> ^[33]
Bronchial asthma	Male, 72	Acute asthmatic attack	Favorable	Italy	Pontillo <i>et al</i> ^[31]
Suspected intractable bronchial asthma	Female, 62	Relapsing polichondritis	Favorable	Japan	Sato <i>et al</i> ^[35]
Allergic asthma	Male, 70	Allergy Cephalosporin use	Favorable	Italy	Santoro <i>et al</i> ^[36]
Asthma	Male, 53	Cocaine	Favorable	United States	Sarkar <i>et al</i> ^[38]
Status asthmaticus	Male, 50	b2 agonist abuse	Favorable	United States	Salahuddin <i>et al</i> ^[32]
Pulmonary embolism					
Pulmonary embolism	Female, 79	Long distance travel Popliteal vein thrombosis	Favorable	United States	Challa <i>et al</i> ^[42]
Pulmonary embolism	Female, 65	Pyelonephritis Emotional stress	Favorable	Italy	Fedele <i>et al</i> ^[41]
Malignancies, invasive procedures/surgery					
Cardiopulmonary bypass	Female	Mitral valve plasty	Favorable	Japan	Itoh <i>et al</i> ^[48]
Rigid bronchoscopy for debridement	Male, 77	Esophageal carcinoma + central airways invasion	Favorable	United States	Guerrero <i>et al</i> ^[50]
Intubation	Female, age not given	Parathyroid surgery (canceled)	Favorable	United States	Mueller <i>et al</i> ^[49]
Bronchoalveolar lavage	Male, 68	Fever and cough productive of sputum, history of tuberculosis	Favorable	South Korea	Ok <i>et al</i> ^[51]
Lung transplantation	Female, 55	End-stage lung fibrosis	Favorable	France	Michel-Cherqui <i>et al</i> ^[47]
Squamous carcinoma	Male, 51	Pulmonary resection	Favorable	South Korea	Lee <i>et al</i> ^[44]
Non-small cell lung cancer	Male, 72	Pulmonary resection	Favorable	Japan	Toyooka <i>et al</i> ^[45]
Lung adenocarcinoma	Male, 59	Heavy smoker, first diagnosis of malignancy with multiple metastases	Favorable	Turkey	Kepez <i>et al</i> ^[46]
Miscellaneous					
Cough	Female, 82	Bad coughing "pill went down the wrong way"	Favorable	United States	Butman <i>et al</i> ^[59]
Dyspnea	Female, 51	Diving (examination)	Favorable	France	Chenaitia <i>et al</i> ^[58]
<i>S. pneumoniae</i> pneumonia	Female, 65	Sepsis	Favorable	Australia	Geng <i>et al</i> ^[55]
Pulmonary edema	Female, 73	Frightening episode	Favorable	Northern Ireland	Daly <i>et al</i> ^[56]
Pulmonary edema	Female, 59	Motor-vehicle collision	Favorable	United States	Ritchie <i>et al</i> ^[57]
Pneumothorax	Female, 64	COPD	Favorable	United States	Kumar <i>et al</i> ^[52]
Pulmonary hypertension	Female, 69	Initiation of intravenous trepostinil	Favorable	United States	Cork <i>et al</i> ^[54]
Pulmonary hypertension	Female, 81	Right ventricular involvement	Favorable	Italy	Citro <i>et al</i> ^[53]
Smoking and "Venus"	Male, 81	Adulterous sexual intercourse	Favorable	Italy	Brunetti <i>et al</i> ^[60]

COPD: Chronic obstructive pulmonary disease. TTC: Tako-Tsubo cardiomyopathy.

lected 37 papers reporting a total of 38 patients (Table 1). As expected, most patients were women ($n = 31$, 81.6%),

mean age was 65 ± 10 years. Outcome was favorable in 100% of cases, and all the patients have been discharged uneventfully in a few days. As for country of origin, 15 studies (40.5%) were conducted in the United States, 5 (13.5%) in Italy, 3 (8.1%) in Japan, 2 (5.4%) each in Brazil, Korea and France, and one (2.7%) each in Poland, Spain, Northern Ireland, Germany, Turkey, Australia, Taiwan, and New Zealand.

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is relatively frequently associated with TTC. In a retrospective analysis of a large cohort of approximately 17000 patients with diagnostic angiographies in Hamburg, Germany, Hertting *et al.*^[20], out of the 32 cases of TTC found that 14 (44%) had COPD or asthma. Since 72% of these patients were taking β -mimetics, the authors postulated that this kind of medication could have acted as preconditioning factor for the cardiomyopathy or aggravated the sympathetic nervous system stress. In fact, several other cases of TTC in patients with β -stimulators abuse have been reported^[21-23]. Multiple admissions for COPD exacerbations may act as a trigger^[24-25], alone or in combination with emotional stressors, *i.e.*, unexpected death of a son^[26], severe financial problems^[27-29], or family dispute^[30].

Asthma

Similarly to COPD, acute asthmatic attack may trigger TTC^[31], and pharmacological treatments may potentiate such an effect^[32-33]. Abuse of nasal decongestants in the course of allergic rhinitis has also been reported^[34]. TTC episodes have also been described in the case of relapsing polychondritis with symptoms of intractable bronchial asthma^[35], allergic asthma secondary to cephalosporin use^[36], abuse of coffee to cope with jet lag^[37], and also concomitant abuse of cocaine^[38]. In the latter case, the TTC cardiotoxic effect could have been potentiated by catecholamines^[39].

Pulmonary embolism

Arterial systemic embolization represents frequent complication during TTC. Mitsuma *et al.*^[40] studied the clinical characteristics and complications of 21 consecutive patients with TTC in Japan. Thromboembolism was found in 3 patients, 1 with ventricular thrombus and 2 with cardioembolic stroke. However, cases of pulmonary thromboembolism have been reported in elderly women as a consequence of acute pyelonephritis^[41], and a popliteal vein thrombosis after a long distance travel^[42].

Malignancies, invasive procedures and surgery

On the one hand, an association of TTC with malignancies has been hypothesized, potentially as a result of paraneoplastic phenomena^[43]. On the other, surgery and invasive procedures represent severe physical stressors capable to trigger TTC onset. Several cases of TTC events in patients with lung malignancies undergoing pulmonary resection have been reported^[44-46], and also after lung

transplantation for end-stage fibrosis^[47] or cardiopulmonary bypass^[48]. Again, other cases were associated with intubation^[49], debridement of central airways neoplastic invasion with rigid bronchoscopy^[50], and even after a simple bronchoalveolar lavage^[51].

Miscellaneous

Several other diseases or condition have been shown to trigger TTC. Among these, pneumothorax^[52], pulmonary hypertension^[53] also after attempt at treatment^[54], pneumonia with sepsis^[55], and pulmonary edema secondary to stressful events^[56,57]. A TTC episode occurred after acute dyspnea secondary to the stress of scuba diving in a 51-year-old woman (at the third immersion, as her level-3 diving examination), has been reported^[58]. Finally, 2 singular episodes of dyspnea occurred in ultraoctogenarians, both of them triggering a TTC episode: A bad coughing since “pill went down the wrong way” in a 82-year-old lady^[59], and a sudden dyspnea occurred in a 81-year-old man during an adulterous sexual intercourse with a young lady^[60].

DISCUSSION

If the question is: “Does an association between respiratory diseases and TTC exist” the answer is yes. On the one hand, patients with severe respiratory diseases, such as asthma or COPD, are exposed to a high risk of developing TTC in the course of critical exacerbations, when they are also compelled to assume high dosages of β -agonists. On the other hand, patients with lung cancer are often exposed to invasive procedures, both diagnostic and surgical, that may be relevant in predisposed subjects. Patients with acute respiratory symptoms or diseases should always be approached with caution in the event of invasive procedures or surgery, keeping in mind the possible acute cardiologic complications.

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COMMENTS

Background

Tako-Tsubo cardiomyopathy (TTC) is a reversible clinical condition mimicking an acute myocardial infarction. Its onset is characterized by multifactorial pathophysiologic mechanisms, and stress may play a crucial role.

Research frontiers

Patients with acute respiratory symptoms or diseases should be approached with caution in the event of invasive procedures or surgery, keeping in mind the possible acute cardiologic complications and the availability of managing abilities.

Innovations and breakthroughs

This is the first study evaluating the association between respiratory diseases and TTC.

Applications

More attention in either suspecting and recognizing TTC, and managing it.

Terminology

TTC is a reversible clinical condition mimicking an acute myocardial infarction. The original Japanese term "tako-tsubo" indicates the particular shape of the end-systolic left ventricle in ventriculography resembling that of the round-bottom and narrow-neck pot used for trapping octopuses.

Peer review

The authors have reviewed the association between respiratory diseases and Tako-Tsubo cardiomyopathy by searching case reports in world wide. They initially described general features of TTC, then discussed about relationship between TTC and respiratory disorders, including chronic obstructive pulmonary disease, asthma, pulmonary embolism, malignancies, invasive procedures, and miscellaneous. This article is well searched and summarized, and may provoke attention of TTC not only to cardiologists but also to pulmonologists and anesthesiologists.

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Long-lasting symptoms and diagnostics in a patient with unrecognized right sided heart failure: Why listening to the heart is so important

Liesbeth C de Vette, Jasper J Brugts, Jacky S McGhie, Jolien W Roos-Hesselink

Liesbeth C de Vette, Jasper J Brugts, Jacky S McGhie, Jolien W Roos-Hesselink, Department of Cardiology, Erasmus MC, 230 3015 CE Rotterdam, The Netherlands

Author contributions: de Vette LC, Brugts JJ, McGhie JS and Roos-Hesselink JW wrote the manuscript, interpreted the clinical data and echocardiographic data, and approved the final version of the manuscript.

Correspondence to: Jolien W Roos-Hesselink, Professor, Department of Cardiology, Erasmus MC, Thoraxcentre's Graven-dijkwal, 230 3015 CE Rotterdam,

The Netherlands. j.brugts@erasmusmc.nl

Telephone: +31-10-8003938 Fax: +31-10-8003939

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central venous pressure, palpable liver, an a cardiac murmur. Based on these findings she should have been referred to a cardiologist in an early stage after which transthoracic echocardiography resulted in the correct diagnosis.

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Abstract

M Ebstein is usually diagnosed in early childhood or adolescence. The young woman in our case complained of fatigue and shortness of breath, on physical examination she showed signs of heart failure with raised central venous pressure, palpable liver, an a cardiac murmur. Based on these findings she should have been referred to a cardiologist in an early stage after which transthoracic echocardiography resulted in the correct diagnosis. In this case the anomaly was missed for many years by different specialists and the patient was treated for liver disease, while she was suffering from liver congestion due to right-sided heart failure.

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Key words: Ebstein; Congenital cardiology; Auscultation; Pregnancy; Liver

Core tip: The young woman in our case complained of fatigue and shortness of breath, on physical examination she showed signs of heart failure with raised

INTRODUCTION

The anamnesis and physical examination have an essential role in the work-up of patients. The current case shows clearly that with thorough examination of the patients' story and physical examination, the majority of medical tests could have been prevented and the correct diagnosis been found earlier.

CASE REPORT

Our patient was born in 1978 after an uncomplicated pregnancy. In childhood, she was seen by a paediatrician because of recurrent tonsillitis and iron deficient anaemia and at the age of seven she experienced an episode of jaundice with urobilinogen in her urine with no clear explanation at that time.

At the age of 13, she complained of headaches and fatigue which were correlated to sinusitis. For pain relief she took paracetamol (acetaminophen) and ibuprofen in high dosages. At age 22, she was analysed at the department of internal medicine because of jaundice. She still complained of fatigue and anorexia now. Thorough

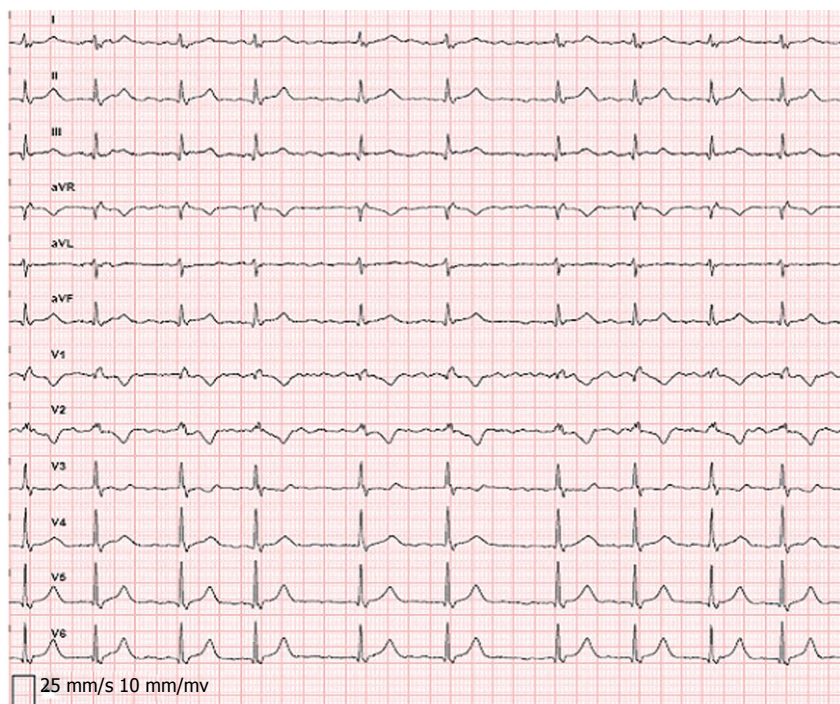


Figure 1 Electrocardiography showing atrial fibrillation, ventricular response 80 beats/min, intermediate axis, right bundle branch block.



Figure 2 Chest X-ray showing marked cardiomegaly caused by right atrium enlargement, cor/thorax ratio 15.5/24.5.

screening revealed a solitary raised bilirubine, no serological evidence of viral infection, and no echographic abnormalities of the liver, gallbladder or biliary ducts. Two years later evaluation was repeated because of persisting elevated bilirubin levels. Due to headaches, she still used 3 g of paracetamol a day for years. The hyperbilirubinemia was now diagnosed as a toxic effect of this medication. After withdrawal of paracetamol, laboratory results did not improve and a liver biopsy was performed. This showed periportal fibrosis without cause. She was referred to the hepatologist for a second opinion and he

found an irregular heart rate and referred her for cardiac consultation for the first time.

We saw a young woman with fatigue, shortness of breath and palpitations on minimal exercise. She could not work due to complaints. Additionally, several attempts to become pregnant were unsuccessful, which was severely stressful for her. Physical examination showed an icteric woman, irregular heart rate of 80 bpm with raised central venous pressure and 4 cm palpable liver. On cardiac auscultation a systolic murmur grade 2 out of 6 at the fourth intercostal space left and a soft diastolic

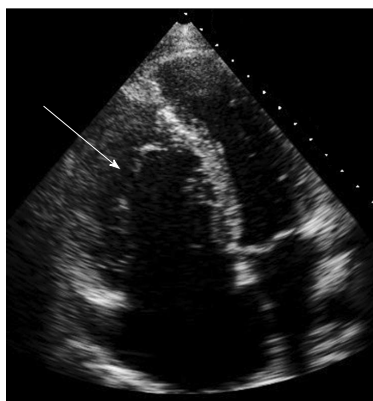


Figure 3 Echocardiography apical 4 chamber view showing the apically displaced tricuspid valve orifice, small right ventricle, large atrialized right ventricle and dilated right atrial.

murmur were heard. The electrocardiography (Figure 1) revealed atrial fibrillation with slow ventricular response and a QRS-complex with right bundle branch block configuration. Chest X-ray showed cardiomegaly (Figure 2). Echocardiography revealed an enlarged right heart with apical displacement of the tricuspid valve (Figure 3). A large part of the right ventricle (RV) was atrialized and in the enlarged RA a large mural thrombus was found (Figure 4). The interatrial septum bulged out towards the compressed left atrium consistent with high RA pressures. Low velocity antegrade flow was found in the main pulmonary artery, in systole and diastole, indicating the RV was functioning as a conduit.

The diagnosis of the congenital defect, Morbus Ebstein, was made which was complicated by intracardiac thrombus. Anticoagulants were started and further analysis showed a perfusion defect in the right lung, consistent with recurrent small pulmonary emboli. After successful treatment of the cardiac thrombus, heart catheterisation was performed (pulmonary artery pressure 13/12; wedge 11 mmHg). As pulmonary hypertension was excluded, fortunately she could be referred for surgical correction. The tricuspid valve was repaired according to Chavaud, and a Carpentier ring was implanted. The atrialized RV was reduced and because of the small dimension of the remaining RV, a partial cavo-pulmonal shunt from superior caval vein to right pulmonary artery (Glenn shunt) was constructed. The post-operative course was uncomplicated and her condition improved considerably. Echocardiography post-operatively showed mild tricuspid-valve insufficiency. One year after surgery, she became pregnant gave birth to a healthy baby. Currently, seven years after surgery, our patient is doing reasonably well and is in NYHA class I - II.

DISCUSSION

Our patient experienced many complaints since childhood which could have been prevented if the correct diagnosis of a congenital heart disease was made earlier. Morbus Ebstein is a rare disorder with a prevalence of



Figure 4 Echocardiography: Low right parasternal view visualizing a large mural thrombus in the large atrialized right ventricle and right atrial (arrow).

about 1 in 50000-200000 and was first described by Ebstein^[1]. In 1866, he published post-mortem analyses of a nineteen-year-old man who presented with dyspnoea, palpitations, systolic murmur, cyanosis and eventually clinical features of heart failure. Obduction showed a deformation of the tricuspid valve with displacement of the effective tricuspid valve orifice towards the apex. Severity of this tricuspid anomaly can vary substantially and can be associated with other defects such as atrial septal defect or patent foramen ovale, which is present in 70%-80% of the patients^[2]. Other associated defects are ventricular septal defects with or without pulmonary atresia, patent ductus arteriosus or aortic coarctation.

M Ebstein is usually diagnosed in early childhood or adolescence. In this case the anomaly was missed for many years by different specialists and the patient was treated for liver disease, while she was suffering from liver congestion due to right-sided heart failure. The frequent use of paracetamol had put the clinicians on the wrong trail in interpreting the elevated liver enzymes, also a liver biops could have been prevented. All her symptoms, the fatigue, shortness of breath and her inability to become pregnant were due to her cardiac situation. The right tract was started after listening to the heart.

COMMENTS

Case characteristics

The young woman in this case complained of fatigue and shortness of breath, on physical examination she showed signs of heart failure with raised central venous pressure, palpable liver, and a cardiac murmur.

Differential diagnosis

Initially the differential diagnosis of fatigue, jaundice and elevated liver enzymes is broad with primarily related to liver disease, medication effects but should incorporate a cardiac evaluation. With the progressive signs of right heart failure on physical examination, the suspicion of a cardiac diagnosis should have become stronger.

Laboratory diagnosis

The hyperbilirubinemia was now diagnosed as a toxic effect of this medication.

Imaging diagnosis

Echocardiography revealed an enlarged right heart with apical displacement of the tricuspid valve.

Treatment

The tricuspid valve was repaired according to Chavaud, and a Carpentier ring was implanted and the atrialized RV was reduced with a partial cavo-pulmonary shunt from superior caval vein to right pulmonary artery (Glenn shunt)

Term explanation

Ebstein is the name of the doctor who first described the anomaly.

Experiences and lessons

The current case underlines the importance of physical examination and main-

taining a broad view to a patient's problem.

Peer review

The case report is well written and addresses common problems with diagnostics of Ebstein anomaly.

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Unreliability of aortic size index to predict risk of aortic dissection in a patient with Turner syndrome

Jan Nijs, Sandro Gelsomino, Fabiana Lucà, Orlando Parise, Jos G Maessen, Mark La Meir

Jan Nijs, Mark La Meir, Cardiothoracic Surgery, University Hospital, 1090 Brussels, Belgium

Sandro Gelsomino, Fabiana Lucà, Orlando Parise, Jos G Maessen, Cardiothoracic Department, Maastricht University Hospital, 6229 HX Maastricht, The Netherlands

Author contributions: Nijs J and Gelsomino S conceived and drafted the article; Parise O and Lucà F contributed to the imaging and revised the manuscript for important intellectual contents; Maessen JG and La Meir M approved the final version of the manuscript to be published.

Correspondence to: Sandro Gelsomino, MD, PhD, Cardiothoracic Department, Maastricht University Hospital, P. Debye-
laan 25, 6229 HX Maastricht,

The Netherlands. sandro.gelsomino@libero.it

Telephone: +31-43-3877070 Fax: +31-43-3875075

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need for careful monitoring and surgical evaluation of the patients even when the ASI is $< 20 \text{ mm/m}^2$ if other significant risk factors are present.

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INTRODUCTION

Turner syndrome (TS) is a relatively common chromosomal disorder, caused by complete or partial X monosomy in some or all cells^[1]. This abnormality is denoted medically as the 45,X karyotype as opposed to the usual 46,XX female karyotype. Many TS patients are actually mosaic, meaning that they have cells with more than one karyotype and occasionally there is mosaicism for cells containing Y chromosome material (Table 1)^[2-4]. Short stature and gonadal dysgenesis are two of the characteristic clinical features of the syndrome, although many organ systems and tissues may also be affected to a lesser or greater extent. However, approximately 50% of karyotypically-proven, asymptomatic women with TS have evidence of abnormal cardiovascular development and most patients die from cardiovascular defects mainly involving the left ventricular outflow tract, left heart and/or aortic hypoplasia. Common congenital defects in surviving girls and adults with TS include bicuspid aortic valve (30%), aortic coarctation (12%) and partial anomalous pulmonary connection (18%)^[5,6]. Nonetheless, the occurrence of aortic dilatation, dissection or rupture is one of major concerns in TS^[7]. The annual incidence of aortic dissection or rupture is 15 cases/100000 for individuals < 20 years of age, 73-78 cases/100000 for women 20-40 years

Abstract

Aortic size index (ASI) has been proposed as a reliable criterion to predict risk for aortic dissection in Turner syndrome with significant thresholds of 20-25 mm/m^2 . We report a case of aortic arch dissection in a patient with Turner syndrome who, from the ASI thresholds proposed, was deemed to be at low risk of aortic dissection or rupture and was not eligible for prophylactic surgery. This case report strongly supports careful monitoring and surgical evaluation even when the ASI is $< 20 \text{ mm/m}^2$ if other significant risk factors are present.

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Key words: Aortic dissection; Aortic aneurysm; Turner syndrome

Core tip: Aortic size index (ASI) has been proposed as a reliable criterion to predict risk of aortic dissection in Turner syndrome. This case report emphasizes the

Table 1 Chromosomal pattern based on karyotyping of women with Turner syndrome

Karyotype	Description	El-Mansoury <i>et al</i> ^[2] 2007 (n = 126)	Gravolt <i>et al</i> ^[3] 1996 (n = 304)	Hook <i>et al</i> ^[4] 1983 (n = 1043)
45,X	Monosomy X	48%	56%	58%
45,X/46,XX	Monosomy X mosaic with normal female sex chromosome complement	23%	17%	15%
46,X,i(Xq)	isochromosome X	13%	11%	15%
46,X,del(X)	deletion chromosome X	-	8%	6%
46,X,r(X)	ring chromosome X	3%	5%	2%
45,X/47,XXX	monosomy X mosaic with triple X chromosome complement	3%	3%	4%
45,X/46,XY	monosomy X mosaic with normal male sex chromosome complement	10%	-	-

old and 50/100000 for older women with TS^[5].

Aortic root enlargement increases the risk of aortic dissection in TS although it is unclear whether such a life-threatening disease is always preceded by progressive dilatation as occurs in marfan syndrome (MS). However, despite connective disorders in which guidelines for monitoring of aortic root dimension and indications for surgical intervention are well established^[8,9], reliable guidelines are lacking for TS and it is uncertain whether any cut-off value of aortic diameter can be used to identify Turner patients at high-risk of aortic dissection.

Furthermore, since body size is a major determinant of normal aortic dimensions, it may not be appropriate to apply standards derived from adult men to a syndrome more common in women and in which small body size is a main characteristic feature.

Aortic size index (ASI), which adjusts the aortic diameter to the body surface area^[4], has been recently introduced as a reliable criterion to predict risk of aortic dissection in TS patients, but its usefulness in this clinical entity is still matter of debate.

We report a case of contained rupture of a dissected aortic arch in a patient with TS who, from the ASI thresholds proposed^[9,10], was deemed to be at low risk of aortic dissection and was not eligible for prophylactic surgery.

CASE REPORT

A 23-year-old woman with TS (45,X karyotype), Graves-Basedow disease and systemic arterial hypertension treated with β -blockers, presented to our hospital facility

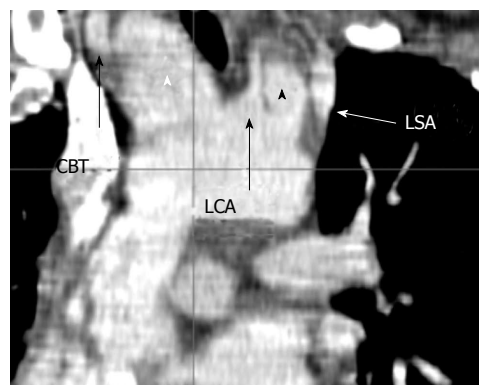


Figure 1 2D Coronal reformatted image. Digital multiplane reformatted image of the aortic arch, depicting the double barrel-shaped contained rupture of the aortic arch, in-between the common brachial trunk (CBT) and the left carotid artery (LCA) (white arrowhead) and LCA and the left subclavian artery (LSA) (black arrowhead).

because of fever unresponsive to antibiotics. She had experienced chest pain 1 mo previously which regressed spontaneously. She had no pain at hospital admission. Blood pressure was 110/82 mmHg.

The patient's height and weight were 160 cm and 82 kg, respectively, with a body surface area of 1.85 m². TS was diagnosed at the age of 14 years after an evaluation for short stature and delay of pubertal development. Since then, the patient underwent yearly computed tomography (CT) which showed any aortic dilatation (the diameter of the ascending aorta at the latest scan before admission was 26 mm).

A CT scan at admission revealed a contained rupture of a dissected aortic arch with two false aneurysms between the common brachial trunk (CBT) and the left carotid artery (LCA), and between the LCA and left subclavian artery (LSA) (Figure 1). A peri-aortic hematoma (Figure 2) originating from the arch was present around the anterior aspect of the ascending aorta. The diameters of the aorta were as follows: ascending aorta 26 mm, arch 30 mm and proximal descending aorta 19 mm. The ascending aortic size index was 14 mm/m². Echocardiography confirmed the diagnosis and revealed the presence of a bicuspid aortic valve and slight valve insufficiency.

A cardio-circulatory arrest with deep hypothermia was planned. After cannulation of the femoral vessels and the axillary artery through a 10-mm graft (Vascutek, Terumo Ltd, Egham, United Kingdom) surgical access was gained through median sternotomy. The ascending aorta was resected and the arch inspected: a rupture was detected between the CBT and the LCA with the tear extending towards the LSA. Because of the hematoma, the CBT could not be encircled or clamped and antegrade cerebral perfusion was conducted *via* the LCA, until the CBT was reconstructed, after which selective antegrade cerebral perfusion *via* the axillary artery was added. Two 12-mm grafts (Gelsoft, Terumo Ltd, Egham, United Kingdom) were anastomosed to the LCA and LSA and a 14-mm graft (Gelsoft, Terumo Ltd, Egham, United Kingdom) was anastomosed to the CBT. A 28-mm graft

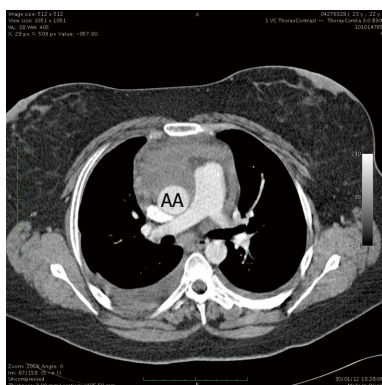


Figure 2 Axial plane computed tomography image of the ascending aorta. Axial image of the ascending aorta at the level of the pulmonary artery bifurcation. The ascending aorta is compressed in an oval shape due to the sub-adventitial spreading hematoma. AA: Ascending aorta.

(Gelweave Terumo Ltd, Egham United Kingdom) was anastomosed to the distal arch. Afterwards, the prosthesis was clamped and the distal body perfusion resumed through the femoral artery. The aortic valve was a “true” bicuspid valve with no raphe and 180° commissural orientation. The aortic root was normal and the effective height of the aortic valve was 9 mm. Therefore, there was no indication for valve and root replacement. After the proximal anastomosis was completed, the supra-aortic vessels were reimplanted on the ascending aorta prosthesis. Cardiopulmonary bypass time was 440 min, aortic cross-clamp time was 180 min and circulatory body arrest time was 20 min. The operation was routinely completed. After an uneventful course, the patient was transferred to the referring hospital on postoperative day 8. Pathologic examination of the aorta revealed very limited myxoid degeneration with no evidence of either fragmentation or separation of the elastic fibers.

DISCUSSION

In TS, it remains unclear whether aortic dissection is preceded by progressive dilatation as it is in connective disorders, and whether the thresholds employed in MS can be safely employed for TS. Nevertheless, a large proportion of these patients are small women and, for this reason, it is not correct to use standards derived from adult men in the general population and, for instance, an ascending aortic diameter even < 5 cm may represent, in these patients, a significant dilatation.

To overcome the body size issue, the ASI has been introduced which adjusts the aortic diameter to body surface area^[10]. Davies *et al*^[7] showed that patients with thoracic aortic aneurysms with ASI < 27.5 mm/m² are at low risk (approximately 4% per year), those with ASI between 27.5 and 42.4 mm/m² are at moderate risk (approximately 8% per year), and those above 42.5 mm/m² are at high risk (approximately 20% per year) of rupture, dissection, and death. Matura *et al*^[8] employed this index in patients with TS demonstrating that subjects with ASI > 20 mm/m² require close cardiovascular surveillance

and those with ASI ≥ 25 mm/m² are at highest risk of aortic dissection.

We presented a case of a 23-year-old TS female with contained rupture of a dissected aortic arch. The ASI in our patient was 14 mm/m² at the level of the ascending aorta. Therefore, following current indications, there was no indication either for surgery or for close surveillance in this patient since the ASI was well below accepted thresholds. Hence, although recent studies^[5] have confirmed that body surface area normalization is the most appropriate approach for determining aortic dilation in TS, in our experience ASI was unable to predict impending aortic dissection and rupture.

A recent study employing mathematical models of aortic disease in TS^[11], showed that growth of the thoracic aorta is dynamic over time and risk factors such as aortic coarctation, bicuspid aortic valves, age, diastolic blood pressure, body surface area and antihypertensive treatment preferentially accelerated growth of the ascending aorta. Unfortunately, this model was not linked to aortic dissection and rupture. However other papers^[3-5] report that bicuspid aortic valve, karyotype 45X, age 20-45 years, and hypertension are factors that confer an increased risk of dissection. All these features were present in the case reported therefore, in our opinion, when one or more of these factors are present, the risk of dissection should be taken into account even with ASI < 20 mm/m², and a close surveillance by a multidisciplinary team (cardiologists, radiologists, cardiac surgeons) should be recommended.

Although a CT scan with contrast is the most widely used diagnostic procedure, recent studies^[12,13] have demonstrated that cardiac magnetic resonance imaging (CMRI) is an important tool for clinical care and it improves risk stratification of TS patients. Indeed, CMRI is outstanding for detection of the degree of aortic dilatation and coarctation that are not visible on echocardiography^[14], but is limited by its high cost and poor tolerability due to claustrophobia and anxiety in some TS patients. Meanwhile, fast scan seeds, low radiation dose and increased anatomic coverage are improving the image quality of cardiac multidetector CT (MDCT) and reducing patient risks in children. Cardiac MDCT is also considered to effectively bridge the gaps among echocardiography and cardiac MRI in children with congenital heart disease. In addition, cardiac MDCT has better cost benefit compared with CMRI.

In conclusion, our experience emphasizes the need for careful monitoring and surgical evaluation of TS patients even when the ASI is small if other significant risk factors are present. Even though this is only a case report, it provides the idea and sounds the alarm that using only an ASI is not sufficient for risk stratification for aortic dissection in patients with TS.

Large prospective studies are needed for risk stratification for aortic dissection in TS in order to identify reliable thresholds to identify patients who may require referral for surgery before life-threatening complications occur.

ACKNOWLEDGMENTS

We gratefully thank Dr. Judith Wilson for the English revision of the paper.

COMMENTS

Case characteristics

A 23-year-old woman with Turner syndrome (TS).

Clinical diagnosis

Fever unresponsive to antibiotics, and chest pain.

Differential diagnosis

Other causes of chest pain, thoracic back pain.

Laboratory diagnosis

Blood, metabolic panel and liver function tests were within normal limits.

Imaging diagnosis

A computed tomography-scan at admission revealed a contained rupture of a dissected aortic arch with two false aneurysms between the common brachial trunk and the left carotid artery and the left carotid artery and the left subclavian artery, respectively. The diameters of the aorta were as follows: ascending aorta 26 mm, arch 30 mm and proximal descending aorta 19 mm. The ascending aortic size index was 14 mm/m².

Pathological diagnosis

Pathologic examination of the aorta revealed very limited myxoid degeneration with no evidence of either fragmentation or separation of the elastic fibers.

Treatment

The patient underwent aortic arch replacement and common brachial trunk, left carotid artery, and left subclavian artery replacement.

Related reports

Aortic root enlargement increases the risk of dissection in Turner syndrome but it is unclear whether aortic dissection is always preceded by progressive dilatation as occurs in Marfan syndrome. Nevertheless, a large proportion of these patients are small women and, for this reason, it is not correct to use standards derived from adult men in the general population and, for instance, an ascending aortic diameter even < 5 cm may represent, in these patients, a significant dilatation.

Term explanation

Aortic size index, which adjusts the aortic diameter to the body surface area, has been recently introduced as a reliable criterion to predict risk for aortic dissection in TS patients but its usefulness in this clinical entity is still a matter of debate.

Experiences and lessons

This case report emphasizes the need for careful monitoring and surgical evaluation of the patients even when the aortic size index is < 20 mm/m² if other significant risk factors are present.

Peer review

This is a potentially interesting case study that describes the limitation in using aortic size index to assess risk of aortic dissection in patients with Turner's syndrome.

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World J Cardiol 2014 June 26; 6(6): 353-516



TOPIC HIGHLIGHT

- 353 Essential hypertension and oxidative stress: New insights
González J, Valls N, Brito R, Rodrigo R
- 367 G-protein-coupled estrogen receptor as a new therapeutic target for treating coronary artery disease
Han G, White RE
- 376 Effect of genetic factors on the association between coronary artery disease and PTPN22 polymorphism
Gloria-Bottini F, Saccucci P, Banci M, Nardi P, Scognamiglio M, Pellegrino A, Bottini E, Chiariello L
- 381 Coronary thrombus in patients undergoing primary PCI for STEMI: Prognostic significance and management
Vecchio S, Varani E, Chechi T, Balducelli M, Vecchi G, Aquilina M, Ricci Lucchi G, Dal Monte A, Margheri M
- 393 Use of intravascular imaging in managing coronary artery disease
Jegere S, Narbutė I, Erglis A
- 405 Role of cardiovascular magnetic resonance in assessment of acute coronary syndrome
Azarisman SM, Teo KS, Worthley MI, Worthley SG
- 415 Clinical disease registries in acute myocardial infarction
Ashrafi R, Hussain H, Brisk R, Boardman L, Weston C
- 424 Timely reperfusion for ST-segment elevation myocardial infarction: Effect of direct transfer to primary angioplasty on time delays and clinical outcomes
Estévez-Loureiro R, López-Sainz A, Pérez de Prado A, Cuellas C, Calviño Santos R, Alonso-Orcajo N, Salgado Fernández J, Vázquez-Rodríguez JM, López-Benito M, Fernández-Vázquez F
- 434 Novel adjunctive treatments of myocardial infarction
Schmidt MR, Pryds K, Bøtker HE
- 444 Invasive strategy in patients with resuscitated cardiac arrest and ST elevation myocardial infarction
Gorjup V, Noc M, Radsel P

- 449** Impact of conditioning hyperglycemic on myocardial infarction rats: Cardiac cell survival factors
Malfitano C, de Souza Junior AL, Irigoyen MC

REVIEW

- 455** Metabolic, autonomic and immune markers for cardiovascular disease in posttraumatic stress disorder
Kibler JL, Tursich M, Ma M, Malcolm L, Greenbarg R
- 462** Antioxidants, inflammation and cardiovascular disease
Mangge H, Becker K, Fuchs D, Gostner JM
- 478** Cardiomyopathies: Evolution of pathogenesis concepts and potential for new therapies
Sisakian H

RETROSPECTIVE STUDY

- 495** Pulmonary arterial hypertension related to human immunodeficiency virus infection: A case series
Araújo I, Enjuanes-Grau C, Lopez-Guarch CJ, Narankiewicz D, Ruiz-Cano MJ, Velazquez-Martin T, Delgado J, Escribano P

OBSERVATIONAL STUDY

- 502** Respiratory modulation of cardiac vagal tone in Lyme disease
Puri BK, Shah M, Monro JA, Kingston MC, Julu POO

SYSTEMATIC REVIEWS

- 507** Therapeutic equivalence in the treatment of hypertension: Can lercanidipine and nifedipine GITS be considered to be interchangeable?
Elliott HL, Meredith PA

CASE REPORT

- 514** Headache: An unusual presentation of acute myocardial infarction
Asvestas D, Vlachos K, Salachas A, Letsas KP, Sideris A

Contents

World Journal of Cardiology
Volume 6 Number 6 June 26, 2014

APPENDIX I-V Instructions to authors

ABOUT COVER Editorial Board Member of *World Journal of Cardiology*, Kaan Kirali, MD, MSc, PhD, MHSc, Head, Professor, Department of Cardiovascular Surgery, Sakarya University, Faculty of Medicine, Education and Research Hospital, Istanbul 34846, Turkey

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Telephone: +86-10-85381891
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Essential hypertension and oxidative stress: New insights

Jaime González, Nicolás Valls, Roberto Brito, Ramón Rodrigo

Jaime González, Nicolás Valls, Roberto Brito, Ramón Rodrigo, Laboratory of Oxidative Stress and Nephrotoxicity, Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Casilla 70058, Chile

Jaime González, Clinical Hospital, University of Chile, Casilla 70058, Chile

Author contributions: González J conducted the critical review of the evidence, wrote and revised the manuscript and designed figures; Valls N conducted the critical review of evidence, wrote the manuscript and designed tables; Brito R conducted the critical review of evidence and revised the manuscript; Rodrigo R wrote and revised the manuscript; all authors read and approved the final version of the manuscript.

Correspondence to: Ramón Rodrigo, MSc, Professor, Laboratory of Oxidative Stress and Nephrotoxicity, Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Independencia 1027, Santiago 7, Casilla 70058, Chile. rrodrigo@med.uchile.cl
Telephone: +56-2-9786126 Fax: +56-2-9786126

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Abstract

Essential hypertension is a highly prevalent pathological condition that is considered as one of the most relevant cardiovascular risk factors and is an important cause of morbidity and mortality around the world. Despite the fact that mechanisms underlying hypertension are not yet fully elucidated, a large amount of evidence shows that oxidative stress plays a central role in its pathophysiology. Oxidative stress can be defined as an imbalance between oxidant agents, such as superoxide anion, and antioxidant molecules, and leads to a decrease in nitric oxide bioavailability, which is the main factor responsible for maintaining the vascular tone. Several vasoconstrictor peptides, such as angiotensin II, endothelin-1 and urotensin II, act through their receptors to stimulate the production of reactive oxygen species, by activating enzymes like NADPH oxidase and

xanthine oxidase. The knowledge of the mechanism described above has allowed generating new therapeutic strategies against hypertension based on the use of antioxidants agents, including vitamin C and E, N-Acetylcysteine, polyphenols and selenium, among others. These substances have different therapeutic targets, but all represent antioxidant reinforcement. Several clinical trials using antioxidants have been made. The aim of the present review is to provide new insights about the key role of oxidative stress in the pathophysiology of essential hypertension and new clinical attempts to demonstrate the usefulness of antioxidant therapy in the treatment of hypertension.

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Key words: Hypertension; Oxidative stress; Endothelial dysfunction; Antioxidants

Core tip: This review focuses on one of the most prevalent diseases worldwide: hypertension, providing new insights about the key role of oxidative stress in the pathophysiology of essential hypertension and new clinical attempts to demonstrate the usefulness of antioxidant therapy in its treatment.

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INTRODUCTION

Hypertension is considered the most important risk factor for the occurrence of cardiovascular disease^[1]. Oxidative stress has gained attention as one of the fundamental mechanisms responsible for the development of hypertension. Reactive oxygen species (ROS) have an important

role in the homeostasis of the vascular wall, hence they could contribute to the mechanism of hypertension^[2-4]. Thus, increased ROS production, and reduced nitric oxide (NO) and antioxidants bioavailability were demonstrated in experimental and human hypertension. Vascular superoxide is derived primarily from NADPH oxidase (NOX) when stimulated by hormones such as angiotensin II (AT-II), endothelin-1 (ET-1) and urotensin II (UT-II), among others. In addition, increased ROS production may be generated by mechanical forces, which increase with hypertension. ROS-induced vasoconstriction results from increased intracellular calcium concentration, thereby contributing to the pathogenesis of hypertension^[2]. Vasomotor tone is dependent upon a delicate balance between vasoconstrictor and vasodilator forces resulting from the interaction of the components of the vascular wall and the blood, and both of them can be altered by oxidative stress. These findings have stimulated the interest on antihypertensive therapies targeted to decrease ROS generation and/or increase NO bioavailability. This review examines the available studies pointing to a role of oxidative stress in the mechanism of production of high blood pressure, as well as the use of antioxidants in the prevention or treatment of this disorder.

PATHOPHYSIOLOGY OF HYPERTENSION

Endothelial dysfunction

Endothelial dysfunction has been implicated in the pathophysiology of different forms of cardiovascular disease, including hypertension. It may be defined as impairment characterized by a shift of the actions of the endothelium toward reduced vasodilation, a proinflammatory state, and prothrombotic setting. These events lead to a state of vascular inflammation, which may be mediated, partly, by ROS formed by activated mononuclear cells.

Vascular oxidative stress and hypertension

Oxidative stress constitutes a unifying mechanism of injury of many types of disease processes, it occurs when there is an imbalance between the generation of ROS and the antioxidant defense systems in the body. The ROS family comprises many molecules that have divergent effects on cellular function. Importantly, many of these actions are associated with pathological changes observed in cardiovascular disease. The effects of ROS are mediated through redox-sensitive regulation of multiple signaling molecules and second messengers^[5-7]. Several studies have demonstrated that essential hypertensive patients and various animal models of hypertension produce excessive amount of ROS^[8-12], and have abnormal levels of antioxidant status^[13], thereby contributing to the accumulating evidence that increased vascular oxidative stress could be involved in the pathogenesis of essential hypertension^[2,3,14]. Recently, it was demonstrated a strong association between blood pressure and some oxidative stress-related parameters^[15]. Other studies show that mouse models with genetic deficient in ROS-

generating enzymes have lower blood pressure compared with wild-type counterparts^[16,17]. In addition, in cultured vascular smooth muscle cells (VSMC) and isolated arteries from hypertensive rats and humans, ROS production is enhanced, redox-dependent signaling is amplified, and antioxidant bioactivity is reduced^[18]. Classical antihypertensive agents such as β -adrenergic blockers, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor antagonists, and calcium channel blockers may be mediated, in part, by decreasing vascular oxidative stress^[19,20].

Sources of ROS in vascular wall

A variety of enzymatic and non-enzymatic sources of ROS exist in blood vessels. The best characterized source of ROS is NOX. In addition to NOX, several other enzymes may contribute to ROS generation, including xanthine oxidase, NO synthase and the mitochondrion.

NOX: NOX is the primary biochemical source of ROS in the vasculature, particularly of superoxide. The kidney and vasculature are rich sources of NOX-derived ROS, which under pathological conditions play an important role in renal dysfunction and vascular damage^[12,21]. This system catalyses the reduction of molecular oxygen by NADPH as electron donor, thus generating superoxide. NOX is up-regulated in hypertension by humoral and mechanical signals. AT-II is the most studied stimulus, but ET-1 and UT-II may also participate in activation of NOX, thereby resulting in increased ROS. Likely the most well-known function of NOX derived superoxide is inactivation of NO to form peroxynitrite, leading to impaired endothelium dependent vasodilation and uncoupling of endothelial nitric oxide synthase (eNOS) to produce additional superoxide^[16,22]. In the vasculature, NOX activation has been strongly associated with hypertension^[23].

Uncoupled endothelial NO synthase: The primary function of eNOS is NO production which regulates vasodilation. Nevertheless, L-arginine and tetrahydrobiopterin (BH₄)-two essential cofactors for its action-deficiency or oxidation are associated with uncoupling of the L-arginine-NO pathway resulting in decreased formation of NO, and increased eNOS-mediated generation of superoxide. NOX is the initial source of ROS. Superoxide combines with NO, which is synthesized by eNOS, to form peroxynitrite^[24]. In turn, peroxynitrite oxidizes and destabilizes eNOS to produce more superoxide^[22,25]. Superoxide also leads to BH₄ oxidation (in fact, BH₄ is highly sensitive to oxidation), which promotes eNOS uncoupling and ROS production.

Xanthine oxidase: Xanthine oxidase is also an important source for oxygen free radical present in the vascular endothelium^[23,26]. It catalyzes the last two steps of purine metabolism. During this process oxygen is reduced to superoxide. There is evidence suggesting involvement of this enzyme in hypertension. Spontaneously hypertensive rats demonstrate elevated levels of endothelial xanthine

oxidase and increased ROS production, which is associated with increased arteriolar tone^[21]. In addition to effects on the vasculature, xanthine oxidase may play a role in end-organ damage in hypertension^[27].

Mitochondrion: The mitochondrion is a major source and target of ROS. Part of the superoxide produced in the intermembrane space may be carried to the cytoplasm^[28]. Ubiquinol or coenzyme Q is a source of superoxide when partially reduced (semiquinone form) and an antioxidant when fully reduced^[29]. Complex I produces most of the superoxide generated by mammalian mitochondria *in vitro*. Complexes II and IV are not normally significant sites of ROS production. Mild uncoupling very effectively decreases the high superoxide production that occurs from complex I. A reduction in antioxidant enzymatic activity in patients with hypertension has been reported^[30].

Role of the vascular wall components

The endothelium senses mechanical and hormonal stimuli. In response, it releases agents that regulate vasomotor function. There is no doubt that endothelium plays a regulatory and protective role by generating vasorelaxing substances. Under some pathophysiological circumstances, endothelium derived vasoconstricting factors, such as ET-1, AT-II, UT-II, superoxide anions, vasoconstrictor prostaglandins and thromboxane A₂, can be released and contribute to the paradoxical vasoconstrictor effects. VSMC are fit not only for short-term regulation of the blood vessel diameter and therefore of blood pressure, but also for long-term adaptation, *via* structural remodeling. ROS mediate many of these pathophysiological processes. The adventitia can contribute to hypertension by either reducing NO bioavailability or participating in vascular remodeling through ROS.

Role of vascular hormones and factors

NO: NO is known to play an important role as a key paracrine regulator of vascular tone. Physiologically, NO inhibits leukocyte-endothelial cell adhesion, VSMC proliferation and migration, and platelet aggregation to maintain the health of the vascular endothelium. Therefore it has many beneficial effects. The decrease in bioavailability of NO in the vasculature reduces vasodilatory capacity and contributes to hypertension. The enzyme that catalyzes the formation of NO from oxygen and arginine is NOS, which in fact is a whole family of enzymes. eNOS is the predominant NOS isoform in the vessel wall. Receptor-mediated agonist stimulation leads to rapid enzyme activation. In addition, shear stress and allosteric modulators are also an important modulator of eNOS activity^[31]. Except the vasorelaxing and antiproliferative properties *per se*, NO plays an important role in antagonizing the effects of AT-II, endothelins and ROS. Nitric oxide diffuses as a gas to the adjacent smooth muscle where it interacts with different receptor molecules such as the soluble guanylyl cyclase. It is accepted

that the normal production of NO plays a crucial role in the maintenance of the physiologic conditions within the cardiovascular system. L-arginine, a substrate for eNOS, seems to be promising in preserving NO formation. However, L-arginine failed to prevent blood pressure increase and left ventricle remodeling due to chronic treatment with L-methyl ester of N-nitro-L-arginin (NAME), an inhibitor of eNOS^[32]. The ACE inhibitor captopril completely prevented NO-deficient hypertension, yet without improving NOS activity. NO also has an ACE down-regulation effect. Thiols protect NO from oxidation by scavenging oxygen-free radicals and by forming nitrosothiols, both effects prolonging NO half-life and duration of NO action^[33,34]. Reduced NO levels can be attributed to elevated levels of ROS. Superoxide combines with NO to form peroxynitrite that oxidizes BH₄ and destabilizes eNOS to produce more superoxide^[22,24,25] thus further enhancing the development of oxidative stress. The balance between NO and AT-II in the vasomotor centers seems to play important role in the regulation of the sympathetic tone.

Renin-angiotensin system: The renin-angiotensin system plays a key role in the development of cardiovascular disease. AT-II is a potent vasoactive peptide that can be formed in vascular beds rich in ACE. When AT-II production increases above normal levels, it induces vascular remodeling and endothelial dysfunction in association with increases in levels of blood pressure. As a potent activator of NOX, AT-II contributes to the production of ROS^[35,36]. In rats and mice made hypertensive by AT-II infusion, expression of NOX subunits, oxidase activity, and generation of ROS are all increased^[37]. AT-II not only increases NOX activity but also upregulates superoxide dismutase activity, possibly to compensate for the increased ROS. In situations where this compensatory effect is efficient, ROS levels may appear normal even in the face of prooxidant. However, when ROS production becomes overwhelming, compensatory mechanisms are inadequate and pathophysiological consequences ensue^[38]. Captopril and enalapril prevented blood pressure rise in young spontaneously hypertensive rats inhibiting ACE. Captopril, probably due to the antioxidant role of its thiol group, had more effective hypotensive effect than enalapril^[39,40]. In contrast, NO not solely antagonizes the effects of AT-II on vascular tone, cell growth, and renal sodium excretion, but also down-regulates the synthesis of ACE and AT₁ receptors. On the other hand, ACE inhibition up-regulates eNOS expression. The ability of AT-II to induce endothelial dysfunction is also due to its ability to down-regulate soluble guanylyl cyclase, thereby leading to impaired NO/cGMP signaling. Recently, it has been proposed that Ca²⁺/calmodulin-dependent protein kinase II is an important molecule linking AT-II, ROS and cardiovascular pathological conditions^[41].

Acetylcholine: In vascular vessels, acetylcholine induces endothelium-dependent dilation *via* production of endo-

thelial factors, mainly NO. NO then diffuses to underlying VSMC, where it induces vascular smooth muscle cell relaxation. The diminution in NO bioavailability will lead to significantly reduced acetylcholine-mediated vasodilation^[39,40]. The consequence of an overall increase in ROS is a reduce bioavailability of NO.

ET-1: Endothelins are potent vasoconstrictor isopeptides produced in different vascular tissues, including vascular endothelium. ET-1 is the main endothelin generated by the endothelium and the most important in the cardiovascular system. When ET-1 is administered in large concentrations, it behaves as a potent vasoconstrictor capable of exerting an array of physiological effects, including the potential to alter arterial pressure. ET-1 mediates its effects through two receptors, ET_A and ET_B. ET_A mediates contractions *via* activation of NOX, xanthine oxidase, lipoxygenase, uncoupled NO synthase, and mitochondrial respiratory chain enzymes. The ET_B induces relaxation on endothelial cells^[42]. Many factors that normally stimulate ET-1 synthesis, (*e.g.*, thrombin, AT- II) also cause the release of vasodilators such as prostacyclin (PGI₂) and/or NO, which oppose the vasoconstricting action of ET-1. It was reported that essential hypertension is characterized by increased ET-1 vasoconstrictor tone, an effect that seems to be dependent on decreased endothelial ET_B-mediated NO production attributable to the impaired NO bioavailability.

UT- II: UT- II is a potent vasoactive peptide^[43], indeed the most potent vasoconstrictor identified. It acts through activation of NOX. The role of UT- II in disease is not well elucidated. The constrictor response to UT- II appears to be variable and highly dependent on the vascular bed examined. Vasoconstriction is not its only effect, because UT receptors have been found in other organs^[44,45]. UT- II has also been shown to act as a potent vasodilator in some isolated vessels^[46].

Norepinephrine: VSMC is innervated primarily by the sympathetic nervous system through adrenergic receptors. Three types of adrenoceptors are present within VSMC: α_1 , α_2 and β_2 . Norepinephrine stimulates VSMC proliferation. In addition, over-expression of inducible NOS increases blood pressure *via* central activation of the sympathetic nervous system, which is mediated by an increase in oxidative stress^[5].

Prostaglandins: PGI₂, another endothelium-dependent vasodilator, relaxes the VSMC. PGI₂ is released in higher amount in response to ligand binding such as thrombin, arachidonic acid, histamine, or serotonin. The enzymes prostaglandin H₂ synthase uses arachidonic acid as a substrate, forming prostaglandin H₂. Prostaglandin H₂ is converted to vasoactive molecules, such as PGI₂. The isoform prostaglandin H₂ synthase-2 may mediate vascular dysfunction in conditions characterized by oxidative stress. Thus, peroxynitrite inhibits the enzymatic activity

of prostacyclin synthase, thereby causing impairment in the PGI₂-mediated vasodilation.

Homocysteine: This molecule may play an important role in the pathogenesis of essential hypertension^[3]. Elevated homocysteinemia diminishes the vasodilation by nitric oxide, increases oxidative stress, stimulates the proliferation of VSMC, and alters the elastic properties of the vascular wall. Thus, homocysteine contributes to elevate the blood pressure^[47]. It is also known that elevated homocysteine levels could lead to oxidant injury of the endothelium^[3]. The correction of elevated homocysteinemia by administration of vitamins B12 and B6 plus folic acid, could be a useful adjuvant therapy of hypertension^[3,48]. However, further controlled randomized trials are necessary to establish the efficacy of these therapeutic agents.

A hypothesis for the role of vascular oxidative stress in hypertension is depicted in Figure 1.

This review has discussed the importance of ROS in the vasculature and its relation to hypertension, but it is important to emphasize the evidence that hypertensive stimuli, such as high salt and AT- II, promote the production of ROS not only in the vasculature, but also in kidney and the central nervous system (CNS) and that each of these sites contributes either to hypertension or to the untoward sequels of this disease^[48].

Role of oxidative stress in kidney

Evidence proposes that ROS play a key role in the pathophysiological processes of several renal diseases; these diseases are considered to be cause and consequence of hypertension. Regarding glomerular alterations, ROS mediates lipoprotein glomerulopathy and other inflammatory glomerular lesions^[49]. A recent study demonstrates that NOX activation and production of ROS through lipid raft clustering is an important molecular mechanism triggering oxidative injury of podocytes induced by homocysteine. This may represent an early event initiating glomerulosclerosis during hyperhomocysteinemia^[50]. Concerning ROS mediated tubulointerstitial injury, one of the mechanisms is the exposure of tubular cells to low-density lipoproteins which may result in tubulointerstitial damage due to ROS production mediated by NOX^[51]. AT- II also plays a pivotal role in the progression of tubulointerstitial injury but also in obstructive nephropathy^[52,53]. It activates NOX and, subsequently, generates superoxide that leads to hypertrophy of renal tubular cells^[54].

There is evidence suggesting that a high-fat diet induces renal inflammation and aggravation of blood pressure in spontaneously hypertensive rats, *via* ROS^[55]. It is also known that the metabolic syndrome is a risk factor for chronic kidney disease (CKD) at least in part independent of diabetes and hypertension *per se*, probably mediated by ROS. Moreover, the onset and maintenance of renal damage may worsen metabolic syndrome features like hypertension, leading to potential vicious cycles^[56].

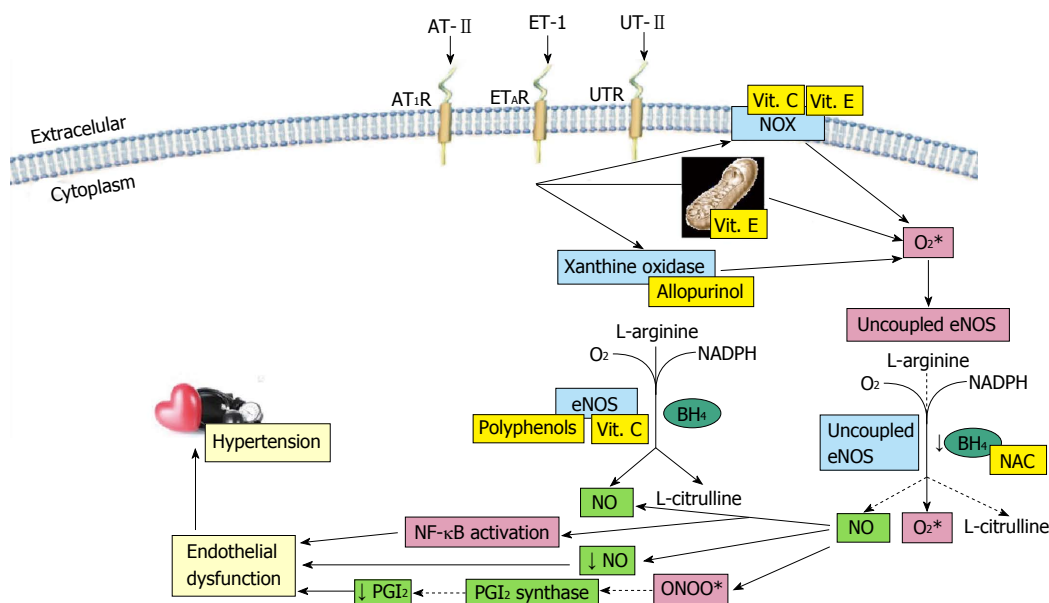


Figure 1 Schematic summary of the role of vascular oxidative stress in the pathogenesis of hypertension and the mechanisms of exogenous antioxidant accounting for anti-hypertensive effects. AT-II: Angiotensin II; AT1R: Type 1 angiotensin II receptor; ET-1: Endothelin 1; ETAR: Type A endothelin receptor; UT-II: Urotensin II; UTR: Urotensin-II receptor; NO: Nitric oxide; eNOS: Endothelial nitric oxide synthase; PGI₂: Prostacyclin; NAC: N-Acetylcysteine; NOX: NADPH oxidase; NF- κ B: Nuclear factor kappa B.

There are several oxidative stress-mediated mechanisms involved in endothelial dysfunction in CKD^[57]. ROS are elevated in CKD and related to endothelium-dependent vascular reactivity and systolic blood pressure^[58]. High ROS and increased level of the endogenous asymmetric dimethylarginine (ADMA) was reported to be a novel risk factor for endothelial dysfunction^[59]. Moreover, high levels of ADMA were reported in CKD and were associated with higher intima-media thickness and cardiovascular events^[60]. In renovascular hypertension, oxidative stress in the ischemic kidney plays a major role in the maintenance of hypertension in two kidney-one clip rats^[61].

Role of oxidative stress in central nervous system

Just like the kidney and the vasculature itself, the sympathetic nervous system (SNS), regulated in the CNS, plays an important role in the pathogenesis of hypertension^[62]. Recent studies strongly suggest that central sympathetic outflow is increased in hypertension^[63]. There is also evidence that increased ROS generation in the brainstem contributes to the neural mechanisms of hypertension in hypertensive rats^[64].

The rostral ventrolateral medulla (RVLM) is the major vasomotor center and is essential for the maintenance of basal vasomotor tone^[65,66]. There are findings that strongly indicate that ROS in the RVLM is increased in stroke-prone spontaneously hypertensive rats and thereby contributes to the neural mechanisms of hypertension through activation of the SNS^[65]. The paraventricular nucleus of the hypothalamus is most likely also involved in the ROS mediated neural mechanism of hypertension^[61,67]. There is evidence that other regions of the brain are also involved in ROS mediated hypertension. These investigations suggest that increased

intracellular superoxide production in the subfornical organ is critical in the development of AT-II-induced hypertension^[68].

Antioxidants in hypertension

This section refers to the antihypertensive role of endogenous and exogenous antioxidants that have demonstrated their ability to alter the blood vessels function and to participate in the main redox reactions involved in the pathophysiology of hypertension.

Vitamin C: Vitamin C is a potent water-soluble antioxidant. On the vascular wall behaves as enzyme modulator exerting up-regulation on eNOS and down regulation of NOX^[69]. Most studies have demonstrated an inverse relationship between plasma ascorbate levels and blood pressure in both normotensive and hypertensive populations^[15]. It has been shown that treatment with antioxidants improves the vascular function and reduces the blood pressure in animal models^[70,71] and in human hypertension^[72,73]. Vitamin C may have favorable effects on vascular dilation, possibly through its antioxidant effects on NO^[74-76].

Nevertheless, there are several small and short-term clinical trials in which the effect of vitamin C supplements on blood pressure have yielded inconsistent findings^[77-82]. The lack of antihypertensive efficacy observed in studies using supplementation with vitamin C alone could be due to the decreased bioavailability of NO under conditions of oxidative stress. It was shown that these effects are mediated in part by the ability of vitamin C to protect BH₄ from oxidation and thereby increase the enzymatic activity of eNOS^[83]. In addition, this uncertain clinical beneficial effect of vitamin C *in vivo* as an antihypertensive agent could be due to the lack of

consideration of their pharmacokinetic properties. It was experimentally determined that the antihypertensive effect of vitamin C is expected to occur at a concentration by $10\mu\text{mo/L}$ ^[75], a plasma level unreachable in humans through oral administration, but that would be required to compete efficiently with the reaction of NO with superoxide. The renal ascorbic acid threshold occurs at vitamin C dose between 60 and 100 mg daily. Plasma is completely saturated at doses of 400 mg daily and higher, producing a steady-state plasma concentration of approximately $80\mu\text{mo/L}$ ^[84]. Thus, the antihypertensive effect may only be active in plasma following vitamin C infusion at high doses.

Vitamin E: This major lipid-soluble antioxidant has received considerable attention for their antioxidant potential. Epidemiological data support a role of high dietary vitamin E intake and a reduced incidence of cardiovascular disease^[57]. Increasing evidence indicates that vitamin E can act as a biological modifier independently of its antioxidant activity. Experimental evidence available shows that vitamin E is capable of dose-dependently regulating mitochondrial generation of superoxide and hydrogen peroxide.

However, intervention trials have not been convincing, with a number of studies demonstrating no beneficial effect of vitamin E on cardiovascular disease outcomes^[85-88]. Moreover, a study using supplementation with vitamin E, either as α -tocopherol or mixed tocopherols, showed a significant increase in blood pressure, pulse pressure and heart rate in individuals with type 2 diabetes^[89]. It should be noted that it is unlikely to achieve sufficiently high concentrations in the vascular microenvironment to interfere effectively with all components of oxidative stress^[90].

Association of vitamins C and E: Ascorbic acid may reduce the α -tocoperoxyl radical and may be required for beneficial vascular effects of α -tocopherol^[91]. In fact, the effect of α -tocopherol seems to be dependent on tissue saturation with vitamin C, and both vitamins may act synergistically to provide optimal conditions for endothelial NO formation^[92]. Thus, the association of vitamins C and E is expected to have an antihypertensive effect probably because this combined therapy provides a reinforcement of their individual properties through a complementary effect^[93].

Despite the biological effects of both vitamin C and E, long-term clinical trials have failed to consistently support their antihypertensive effects in patients at high cardiovascular risk. Some short-term trials have shown that supplemental antioxidant vitamin intake lowers blood pressure^[78,81,82,94] but the majority of clinical long-term trials did not find any antihypertensive effects of antioxidant vitamins. However, most of these studies lack rigorous exclusion criteria in the selection of subjects to avoid the influence of confounders^[95]. It deserves special mention that regarding cohorts included in large trials,

most subjects had irreversible cardiovascular disease.

Allopurinol: Xanthine oxidase is an important source of ROS in the vascular endothelium^[24]. It catalyzes the last two steps of purine metabolism, producing uric acid. Xanthine oxidase activity is associated with an increasing arteriolar tone and therefore, hypertension^[96,97]. Xanthine oxidase inhibitors such as allopurinol have shown marked improvements in endothelial function in various cohorts at risk of cardiovascular events. Treatment with allopurinol result in reduction of blood pressure in adolescents^[98], spontaneously hypertensive rats^[99] and patients with CKD^[100]. Nevertheless, most of the evidence so far comes from smaller mechanistic studies, and the few large randomized controlled trials have not shown significant mortality benefit using these agents^[101].

Selenium: Selenium is an essential trace element and an integral part of many proteins with catalytic and structural functions. It exerts an antioxidant function mainly in the form of selenocysteine residues, an integral constituent of ROS-detoxifying selenoenzymes, such as glutathione peroxidase (GSH-Px), thioredoxin reductases (TR) and selenoprotein P^[102]. Maintenance of full GSH-Px and TR activity by adequate dietary selenium supply has been proposed to be useful for the prevention of several cardiovascular disorders^[83]. In addition, selenium is capable of preventing the union of nuclear factor kappa B (NF- κ B) to its nuclear response elements in DNA^[103]. NF- κ B has a key role in inflammation and production of ROS. The inhibition of NF- κ B is presumed to be the result of the binding of the selenium to the essential thiols of this transcription factor^[104].

Its antioxidant properties have been documented in several trials^[103,105-110]. Selenium at low doses can provide significant protection of the human coronary artery endothelium against damage by oxidative stress^[102]. In an animal model, dietary supplementation with selenium was associated with lower levels of cardiac oxidative damage and increased antioxidant expression, as well as a reduction in disease severity and mortality in spontaneously hypertensive rats^[111]. A reduced selenium concentration in hypertensive pregnancies has been associated with a diminution of GSH-Px activity^[112]. Thus it is reasonable to say that deficiency of selenium might be an underestimated risk factor for the development of high blood pressure^[113].

N-acetylcysteine: The antioxidant N-acetylcysteine (NAC), a sulfhydryl group donor, improves renal dysfunction and decreases arterial pressure and renal injury in salt-sensitive hypertension^[114]. The inhibition of oxidative stress in hypertensive states probably contributes to the therapeutic effects of NAC, an effect likely mediated by an NO-dependent mechanism^[115]. This protective mechanism is exerted by prevention of BH₄ oxidation by the increased superoxide^[116]. In addition, this molecule can protect against oxidative damage inhibiting lipid per-

oxidation and scavenging ROS^[117,118].

Polyphenols: Polyphenols are the most abundant antioxidant in diet. They can act as ROS scavengers, iron chelators, enzyme modulators^[119,120], and possibly enhancing the production of NO^[121,122]. In humans, after the consumption of polyphenols, circulating NO concentration increases^[123]. Polyphenols also increase glutathione, and inhibit ROS-producing enzymes such as NADPH and xanthine oxidases. These pathways lead to improved endothelial function^[124]. However, some studies have shown increased blood pressure by association of polyphenols with vitamin C^[125].

Diet: There is sufficient evidence to suggest that dietary approaches may help to prevent and control high blood pressure. There are dietary approaches regarding the prevention and management of hypertension: *i.e.*, moderate use of sodium, alcohol, an increased potassium intake, plant fibers, calcium, and foods like salmon, nuts, wine, among others^[126]. In a Mediterranean population with an elevated fat consumption, a high fruit and vegetable intake is inversely associated with blood pressure levels^[127]. Short-term studies indicate that specialized diets may prevent or ameliorate mild hypertension, most notable are the Dietary Approaches to Stop Hypertension (DASH) diet, which is high in fruits, vegetables, and low-fat dairy products^[128]. It has been reported that a low sodium DASH diet is effective in reducing blood pressure in hypertensive patients, particularly in those taking antihypertensive medications^[129]. In addition, DASH diet had significant beneficial effects on cardiovascular risk^[130-132]. In overweight or obese persons with above-normal blood pressure, the addition of exercise and weight loss to the DASH diet resulted in even larger blood pressure reductions, greater improvements in vascular and autonomic function, and reduced left ventricular mass^[133,134].

Pharmacological attempts aimed to reduce blood pressure with antioxidant therapies

Recent advances in understanding the complexity of redox signaling in the vascular system points to a central role of oxidative stress in the pathogenesis of vascular dysfunction. This is how hypertension is associated with impaired endothelium-dependent vasodilation with inactivation of endothelium-derived nitric oxide by oxygen free radicals. In this regard, it has arisen a growing interest concerning the therapeutic possibilities to target ROS in the management of essential hypertension.

In support of this view, epidemiological studies suggest that individuals with higher antioxidant intake have reduced cardiovascular risk. In fact, population-based observational studies have shown an inverse association between diverse plasma antioxidant concentrations, obtained by dietary intake, with blood pressure^[113,135], providing justification for trials evaluating antioxidant supplementation as adjunct anti-hypertensive therapy favoring blood pressure reduction.

Antihypertensive effects of vitamin C were hypothesized as early as 1946^[136], and it has been proven that vitamin C enhances endothelial function through effects on nitric oxide production^[75]. Most studies have demonstrated an inverse relation between vitamin C plasma levels and blood pressure, in normotensive and hypertensive populations^[27,137]. However, evidence for blood pressure-lowering effects of vitamin C in clinical trials is still inconsistent. Nevertheless, laboratory^[138,139] and human studies^[140,141] have established biological plausibility for a clinical use of antioxidants concerning hypertension.

Taddei *et al.*^[142] made one of the first trials in 1998, where patients with essential hypertension received intra-arterial infusion of vitamin C, and showing that in essential hypertensive patients vitamin C significantly increased the vasodilation effect of the muscarinic agonist, acetylcholine, indicating that antioxidant vitamin C improves endothelium-dependent vasodilation in hypertensive patients. As ratifying evidence, On *et al.*^[143] in 2002 conducted a study that achieved similar results on endothelium dysfunction, using vitamin C as an adjunctive therapy to Amlodipine.

Despite the evidence points to the use of vitamin C as an adjunct in the treatment on essential hypertension, there is still lack of long-term studies that support its use. Up to date there are few trials that have used chronic supplementation. In a small randomized, double-blind controlled trial^[144], patients were followed for 8 mo and were randomized to receive 500, 1000 and 2000 mg of vitamin C once daily. Results of this study showed a significant diminution of both, mean systolic blood pressure and diastolic blood pressure, with no differences between the increasing doses of vitamin C. Additionally, these effects were only seen during the first month of supplementation, suggesting only a short term benefit. Besides this, other trial aimed to study the effects of ascorbic acid on ambulatory blood pressure in elderly patients, showing that chronic supplementation of vitamin C (600 mg/daily) markedly reduced systolic blood pressure and pulse pressure in ambulatory patients^[145]. Furthermore, this was accompanied by decreases of oxidative stress biomarkers such as levels of 8-isoprostane and malondialdehyde.

The strongest evidence of the possible role of vitamin C on hypertension treatment was handed by a recent meta-analysis that included twenty-nine trials, concluding that in short-term trials, vitamin C supplementation reduces systolic and diastolic blood pressure. But it also highlights that long-term trials on the effects of vitamin C on blood pressure and clinical events are still needed to elucidate its true benefit^[146].

Because supplementation made only with vitamin C has achieved inconsistent clinical outcomes, the scientific rational approach has led to the suggestion that the combined intake of antioxidants could achieve better clinical results. To prove this concept, a small randomized double-blind placebo-controlled trial was made including 38 subjects, 21 hypertensive and 17 normotensive^[81]. Groups

Table 1 Clinical trials accounting for strategies using antioxidants in essential hypertension

Details of Study	Study	n	Results	Ref.
Intrabrachial vitamin C (2.4 mg/100 mL forearm tissue per minute)	Randomized placebo-controlled trial	28	In hypertensive patients but not in control subjects, vitamin C increased the impaired vasodilation to acetylcholine	[141]
Intra-arterial infusion of vitamin C at 24 mg/min for 10 min	Randomized trial	16	Forearm blood flow response to acetylcholine was significantly enhanced with intra-arterial infusion of vitamin C in hypertensive group before antihypertensive treatment	[142]
Oral administration of 500, 1000 or 2000 mg of vitamin C once daily	Randomized double-blind, placebo-controlled trial	31	Significant diminution of mean systolic blood pressure and diastolic blood pressure, with no differences between the increasing doses of vitamin C	[143]
Chronic supplementation of 600 mg/daily of vitamin C	Randomized placebo-controlled trial	24	Reduced systolic blood pressure and pulse pressure in ambulatory elderly patients, but not in adult group	[144]
Included 29 trials of vitamin C supplementation	Meta-analysis	-	In short-term trials, vitamin C supplementation reduces systolic and diastolic blood pressure	[145]
Crossover design Placebo or antioxidant combination: 200 mg zinc 500 mg vitamin C 600 mg vitamin E 30 mg of β -carotene	Randomized double-blind placebo-controlled trial	38	Combined therapy of antioxidants showed that systolic blood pressure fell significantly on hypertensive subjects	[80]
Oral supplementation: 1 g vitamin C 400 UI vitamin E or Placebo for 8 wk	Randomized double-blind, placebo-controlled, crossover study	30	Treatment with vitamins C and E has beneficial effects on endothelium-dependent vasodilation in untreated essential hypertensive patients	[153]
Oral supplementation: 1 g vitamin C 400 UI vitamin E or Placebo for 8 wk	Randomized double-blind placebo-controlled trial	110	Specific association between oxidative-stress related parameters and blood pressure Patients with essential hypertension had significantly lower systolic, diastolic and mean arterial blood pressure	[146]
ACEI plus NAC (600 mg three times a day) or ACEI only	Randomized controlled trial, crossover study	18	Significant decrease in systolic and diastolic blood pressure with the combination of ACEI and NAC compared to ACEI-only	[147]
Standard therapy or Melatonin plus antihypertensive standard therapy	Randomized controlled trial	170	Combined therapy had better outcomes than standard therapy alone on essential hypertensive patients	[148]
Intra-arterial administration: NAC (48 g/min) or vitamin C (18 mg/min)	Cross-over randomized study	30	The intra-arterial administration of NAC had no effect on endothelium-dependent vasodilation Intra-arterial vitamin C improved endothelium-dependent vasodilation	[151]
Coenzyme Q10, 100 mg twice daily or placebo	Randomized, double-blind, placebo-controlled crossover study	30	There was not statistically significant reductions systolic or diastolic blood pressure	[150]
Vitamin C supplement daily Either 50 mg or 500 mg, for 5 yr	Randomized double-blind controlled trial	244	Neither systolic nor diastolic blood pressure was significantly related with the serum vitamin C concentration	[152]

ACEI: Angiotensin-converting enzyme inhibitors; NAC: N-Acetylcysteine.

were assigned to receive in a crossover design placebo or a combination of antioxidants consisting of zinc, ascorbic acid, alpha-tocopherol and beta-carotene daily for 8 wk. Although it was a short-term following, this combined therapy of antioxidants showed that systolic blood pressure fell significantly on hypertensive subjects while been on the antioxidant phase compared with placebo. Additional evidence was given by another study aimed to evaluate the effect of short-term combined treatment with antioxidants vitamin C and E^[95]: 30 essential hypertensive patients were assigned randomly either to vitamin C plus vitamin E or placebo for 8 wk. Results showed that parameters of flow-mediated dilation of the brachial artery and central pulse wave velocity were significantly improved after antioxidant supplementation, concluding that treatment with vitamins C and E has beneficial effects on endothelium-dependent vasodilation in untreated essential hypertensive patients.

Following the same consideration, recently a randomized double-blind placebo-controlled clinical trial was conducted to test the hypothesis that oral administration of vitamin C and E together causes decrease in blood pressure in patients with mild-to-moderate essential hypertension, 110 men with recent diagnosis of grade 1 essential hypertension were randomly assigned to receive either vitamin C (1 gr) plus Vitamin E (400 UI) daily or placebo for 8 wk. The results of this study, showed for the first time a specific association between oxidative-stress related parameters and blood pressure. Following administration of vitamins C plus E, patients with essential hypertension had significantly lower systolic, diastolic and mean arterial blood pressure^[147].

According to the theoretical possibility of the role of antioxidants, further trials have been performed using different compounds with antioxidant activity. This is how Barrios *et al*^[148] in 2002 conducted a patient cross-

over study with the aim to investigate the potential effect of NAC added to the Angiotensin-converting enzyme inhibitors (ACEI) antihypertensive action. A significant decrease in systolic and diastolic blood pressure was achieved with the combination of ACEI and NAC compared to ACEI-only period^[148].

A more recent study tried the use of melatonin to evaluate its effectiveness as an adjunct for a combined treatment adding melatonin to standard anti-hypertensive drugs^[149]. This study showed that combined therapy had better outcomes than standard therapy alone on essential hypertensive patients.

Although there is objective compelling evidence supporting the use of antioxidants in the management of hypertensive patients, there are also several studies that have not shown beneficial effects. As an example: Vitamin E^[150], Coenzyme Q10^[151], NAC^[152] and vitamin C^[153] have failed to obtain beneficial effects on clinical settings.

A summary of the antioxidant approaches as clinical interventions on essential hypertension is presented on Table 1.

CONCLUSION

There is considerable evidence supporting the view that oxidative stress is involved in the pathophysiology of hypertension. ROS are mediators of the major physiological vasoconstrictors, increasing intracellular calcium concentration. In addition, superoxide reduces the bioavailability of NO and enhances superoxide production *via* uncoupled eNOS, further enhancing oxidative stress, a major factor of hypertension.

Antioxidant therapy can curtail the development of hypertension in animal models, but remains controversial in humans. Possible confounding factors in patients include co-existing pathologies and treatments, lack of selection of treatments according to ROS levels, among others. However, the dietary intake of antioxidants and polyphenols could have an effect in the primary prevention or reduction of hypertension. Despite existing molecular basis and *in-vitro* evidence supports the use of diverse antioxidants, clinical evidence continues to be controversial. It is necessary to collect efforts in performing basic/clinical trials that augment the current, which could eventually help to elucidate the role of antioxidants as novel therapy for essential hypertension. Also available data lead us to think that antioxidants may not play the same role in different stages of disease, suggesting that supplementation could be only beneficial during the phase of endothelial dysfunction, which precedes an established vascular damage. In this setting antioxidants would be more likely to have a role on early stages of hypertension with the potential to reverse or counteract deleterious effects of ROS. In contrast, it should not be expected an anti-hypertensive effect in patients having an advanced state of cardiovascular disease, in which chronic damaging effects of oxidative stress may be unreachable for antioxidant approach.

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WJC 6th Anniversary Special Issues (2): Coronary artery disease

G-protein-coupled estrogen receptor as a new therapeutic target for treating coronary artery disease

Guichun Han, Richard E White

Guichun Han, Women's Health Division, Michael E DeBakey Institute, Department of Physiology and Pharmacology, College of Veterinary Medicine and Biomedical Sciences, Texas A and M University, College Station, TX 77843, United States

Richard E White, Department of Biomedical Sciences, Georgia Campus-Philadelphia College of Osteopathic Medicine, Suwanee, GA 30024, United States

Author contributions: Han G and White RE contributed equally to this paper; all authors have approved the final review of this paper.

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Correspondence to: Guichun Han, MD, PhD, Women's Health Division, Michael E DeBakey Institute, Department of Physiology and Pharmacology, College of Veterinary Medicine and Biomedical Sciences, Texas A and M University, MS 4466, College Station, TX 77843, United States. ghan@cvm.tamu.edu
Telephone: +1-979-8456099 Fax: +1-979-8456544

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Abstract

Coronary heart disease (CHD) continues to be the greatest mortality risk factor in the developed world. Estrogens are recognized to have great therapeutic potential to treat CHD and other cardiovascular diseases; however, a significant array of potentially debilitating side effects continues to limit their use. Moreover, recent clinical trials have indicated that long-term postmenopausal estrogen therapy may actually be detrimental to cardiovascular health. An exciting new development is the finding that the more recently discovered G-protein-coupled estrogen receptor (GPER) is expressed in coronary arteries-both in coronary endothelium and in smooth muscle within the vascular wall. Accumulating evidence indicates that GPER activation dilates coronary arteries and can also inhibit the proliferation and migration of coronary smooth muscle cells.

Thus, selective GPER activation has the potential to increase coronary blood flow and possibly limit the debilitating consequences of coronary atherosclerotic disease. This review will highlight what is currently known regarding the impact of GPER activation on coronary arteries and the potential signaling mechanisms stimulated by GPER agonists in these vessels. A thorough understanding of GPER function in coronary arteries may promote the development of new therapies that would help alleviate CHD, while limiting the potentially dangerous side effects of estrogen therapy.

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Key words: G-protein-coupled estrogen receptor; Coronary arteries; G-1; Atherosclerosis; Estrogen

Core tip: A continuing controversy in cardiology is the impact of estrogen on coronary arteries. This review provides the latest information on the discovery of a novel estrogen receptor in these vessels: the G-protein-coupled estrogen receptor (GPER). Recent findings demonstrate that GPER activation induces coronary artery relaxation and attenuates the proliferation and migration of coronary smooth muscle cells. Thus, GPER appears to be a promising, novel pharmacological target that could increase coronary blood flow in diseased arteries and prevent or reverse the progression of coronary atherosclerotic disease, and do so with potentially fewer dangerous side effects associated with traditional estrogen therapy.

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INTRODUCTION

Evidence that ovarian hormones can influence blood flow in other vascular beds was provided well over a century ago^[1]; yet the overall impact of estrogens on cardiovascular function is still quite controversial. For example, there are conflicting reports regarding the potential therapeutic uses of estrogens to alleviate or prevent cardiovascular disease, as clinical trials have found both preventative^[2] and deleterious^[3] effects of conjugated equine estrogens (a mixture of at least 10 different estrogens) on coronary heart disease (CHD) in menopausal women. More directly, 17 β -estradiol (E2), the predominant and most potent circulating estrogen in premenopausal women, has clearly defined effects on blood vessel function. For example, numerous studies have demonstrated that estradiol dilates coronary arteries from humans or other species, and does so by specifically targeting both vascular smooth muscle (VSM) and endothelial cells. Estradiol increases coronary blood flow in intact hearts^[4,5], dilates coronary arteries *in situ*^[6-8], and relaxes coronary arteries isolated *in vitro*^[9-12]. Such studies strongly suggest an inherent therapeutic potential of E2 for alleviating or possibly even preventing coronary insufficiency. As a caution, however, it should be noted that we have demonstrated that E2 can also constrict coronary arteries under experimental^[13] or pathological^[14] conditions *via* a non-genomic mechanism. Thus, E2, a “well-known vasodilator”, is actually a powerful, multifunctional vasoactive hormone whose signaling mechanisms are heterogeneous and complicated, and whose overall physiological effect on coronary arteries apparently depends upon the biochemical disposition of cells located in the vascular wall.

In addition to directly modulating coronary artery function, estrogens may also slow the progression of coronary atherosclerotic disease. Young women are normally protected from significant atherosclerotic plaque accumulation in coronary arteries. Menopause, however, brings unhealthy changes in plasma lipoproteins [*i.e.*, increased low-density lipoproteins (LDLs), and decreased high-density lipoproteins (HDLs), respectively], whereas postmenopausal estrogen replacement therapy (ERT) reverses these potentially harmful changes by decreasing LDL and increasing HDL^[15-17]. More specifically, the phenolic ring structure of estrogens appears to exert an antioxidant effect that may attenuate oxidation of lipids^[18,19] and LDLs^[20-22], and it is oxidized LDLs are accumulated by macrophages in the early stages of atherosclerosis leading to foam cell formation and atherogenesis. Further, E2 can lower the activity and expression of vascular NADPH oxidase, a potent source of reactive oxygen species^[23-25]. Thus, there is increasing evidence that an antioxidant effect of estrogen may attenuate the development of coronary atherosclerosis to help preserve optimal cardiac function throughout a woman's reproductive years^[26].

One might expect oxidant stress to increase during menopause as the antioxidant influence of estrogen begins to wane. Indeed, menopausal women do exhibit greater levels of oxidative stress compared to women of

childbearing age^[27]. In light of the demonstrated beneficial effects of E2 on plasma lipoproteins, it is puzzling that recent clinical trials continue to indicate a mostly deleterious effect of long-term postmenopausal hormone therapy on cardiovascular health. A potential reason for this apparent contradiction is that ERT, while reducing total LDL cholesterol level, does not reduce the number of LDL particles—which, become smaller, therefore more atherogenic^[28]. In addition, we have demonstrated that E2 can actually promote oxidative stress in coronary arteries by enhancing the activity of uncoupled nitric oxide synthase expressed in VSM cells^[13]. Thus, it seems likely that the influence of estrogens on cardiovascular health cannot be explained solely by an antioxidant effect on plasma lipids or blood vessels.

In summary, there is convincing evidence that estrogens can exert powerful effects on both the structure and function of coronary arteries, and that E2 can produce both beneficial and harmful effects on cardiovascular function. As our appreciation of estrogen grows, so should the potential to develop estrogen-like compounds as novel therapeutic agents to prevent or treat CHD. The current challenge remains to fully elucidate the cellular/molecular basis of estrogen action on coronary arteries. At present, however, our understanding of these mechanisms is far from complete, and is at times quite controversial. An important first step is to understand the nature of the specific estrogen receptor molecules that bind E2 and thereby initiate the complicated process of estrogen signaling in coronary arteries.

G-PROTEIN-COUPLED ESTROGEN RECEPTOR AND CORONARY ARTERY TONE

For years it was believed that E2 action on coronary arteries was mediated only *via* activation of one or both of the “classic” estrogen receptors (ER), ER α or ER β . For example, both ERs are expressed in human coronary artery smooth muscle cells (CASMC); but it is ER α that appears to play a great role in mediating acute responses to E2 in these cells *via* increased nitric oxide (NO) generation^[29]. Similarly, porcine coronary arteries were relaxed *via* a NO-dependent mechanism *in vitro* by an ER α -selective agonist, whereas an ER β -selective agonist appeared to induce an NO-independent relaxation response^[30]. It is the ER α subtype that helps protect against ischemia/reperfusion injury in rabbit hearts^[31], whereas ER β did not impact ischemic tolerance significantly^[32]. In addition, optimal functioning of coronary artery endothelial cells is abrogated in mice lacking expression of ER α ^[33]. On the other hand, studies of coronary arteries obtained from human females indicated that it was ER β , which was associated with coronary calcification and atherosclerosis, not ER α ^[34]. These studies suggest that although ER α and ER β are both expressed in coronary arteries, it is ER α that may play the greater role in an acute vasodilatory response to E2 and possibly cardio-

protection as well.

It is the dependency of estrogen on ER α and ER β activation that has limited the potential use of estrogen as a therapeutic agent to alleviate coronary artery dysfunction. Estrogens are powerful endocrine hormones, and these endocrine effects (*e.g.*, secondary sex characteristics) are mediated primarily by classic ERs which are expressed in most cell types. In terms of specifically treating cardiovascular disease the need is to pharmacologically mimic the beneficial effects of estrogens on coronary arteries while minimizing the oftentimes unwanted endocrine side effects in other tissues. Selective ER modulators (SERMs)-agents which act as ER agonists in some target tissues but not in others-have had some success in meeting this therapeutic ideal, but their use still falls short of providing protection against coronary artery disease without endocrine and other side effects. An exciting new development in this important field of investigation is the discovery of a novel G-protein-coupled estrogen receptor (GPR30, now GPER). GPER activation constitutes an acute, non-genomic mechanism of estrogen action that may avoid many, if not most, potential effects of estrogen on endocrine target tissues. Discovery of GPER expression and function in blood vessels has opened the potential for this receptor to serve as a novel therapeutic target.

What was often noticed, yet seldom appreciated, was that some commonly employed ER “antagonists” did not always attenuate the vasodilatory effect of E2 on coronary arteries, and sometimes actually exhibited a direct vascular action themselves. For example, ICI182,780 (fulvestrant) has long been considered a “pure” ER α /ER β antagonist, an estrogen receptor down regulator (SERD); however, this ER blocker did not significantly affect E2-induced coronary dilation or blood flow in canine hearts^[8]. These studies suggested a vasodilatory effect of E2 on coronary arteries that was not mediated by either of the classic ERs. Moreover, we^[35] and others^[36] have demonstrated that ICI182,780 can itself relax porcine coronary arteries *in vitro* (*i.e.*, an effect independent of ER α or ER β activation). In addition, ICI182,780 does not inhibit coronary artery relaxation induced by the SERM raloxifene, which acts directly on CASC^[37]. Taken together, these findings strongly suggest that E2 (and possibly ICI182,780 and raloxifene as well) can relax coronary arteries *via* a mechanism that does not involve activation of ER α or ER β . Interestingly, it is now known that ICI182,780 and raloxifene are agonists for GPER^[36,38-40].

GPER was originally cloned in 1997 from breast cancer^[41] and other cells, including endothelial cells^[42,43], and was found to exhibit the canonical seven transmembrane spanning regions common to all G-protein-coupled receptors. GPER is expressed in the heart and in a variety of blood vessels^[44,45], and GPER mRNA and protein have been detected in porcine and human CASC^[46-48]. Functionally, studies employing the selective GPER agonist, G-1, have indicated a vasodilatory response to G-1 stimulation. Acute treatment with G-1 relaxes rat aorta^[49], rat mesenteric arteries^[50], human internal mammary arteries^[50], and rat carotid arteries^[40,51], whereas infusion

of G-1 induces an acute decrease in arterial pressure^[50]. In addition, recent studies have demonstrated that G-1 induces an acute relaxation response in porcine coronary arteries^[36,46]. These pharmacological studies have been bolstered by use of G15, a selective GPER antagonist, which attenuates vascular relaxation induced by E2^[52] or G-1^[46,52]. Thus, there is increasingly consistent evidence obtained from both protein and pharmacological studies that activation of GPER exerts a vasodilatory effect, particularly in coronary arteries. These findings also substantiate the putative role of GPER in mediating E2-induced coronary artery relaxation.

Relatively few studies have investigated the signaling mechanisms mediating GPER-induced coronary artery relaxation, and these reveal that GPER can relax coronary arteries acutely *via* diverse mechanisms. There is good agreement that G-1 induces a maximal 30%-40% relaxation of porcine epicardial coronary arteries *in vitro* (after taking into account the effect of ethanol or dimethylsulfoxide vehicle on these vessels); however, this relaxant effect of G-1 may be endothelium-dependent or -independent. Meyer *et al.*^[36] report that activation of GPER induces a rapid relaxation of coronary arteries, and that this process is mediated by NO release from endothelial cells. In contrast, Yu *et al.*^[46] provide evidence for a direct relaxant effect of G-1 on CASC, which is bolstered by patch-clamp experiments demonstrating that G-1 opens calcium-activated potassium (BK_{Ca}) channels in isolated porcine and human CASC (*i.e.*, in the absence of endothelium). In this later study G-1-induced relaxation was inhibited by blocking these same potassium channels, but was not attenuated by inhibiting NO or cyclic guanosine monophosphate synthesis. Thus, there appears to be a redundancy of mechanisms mediating GPER-induced coronary artery relaxation: indirect (endothelial cells) and direct (CASC), and may or may not involve production of NO. Because E2 is a very lipophilic hormone that easily traverses biological membrane, it is likely that both cell types mediate GPER-induced coronary artery relaxation *in vivo* (unless there is significant endothelial dysfunction in which case the direct action on CASC should predominate). In addition, there is significant expression of aromatase in both endothelial and VSM cells allowing estrogen to be synthesized directly within the coronary artery wall (*i.e.*, independent of plasma E2 levels)^[53].

As mentioned above, raloxifene is also an agonist for GPER and stimulates an endothelium-independent relaxation of porcine coronary arteries^[37]. In this study the presence of high extracellular potassium (*i.e.*, 30 or 60 mmol) significantly reduced raloxifene-induced relaxation, as did iberiotoxin, a highly specific inhibitor BK_{Ca} channels. In contrast, inhibiting ER α or ER β with ICI182,780 had no effect on the response to raloxifene. Further patch-clamp studies demonstrated that raloxifene elevates iberiotoxin-sensitive outward currents in isolated CASC. Thus, the endothelium-independent relaxation effect of raloxifene on porcine coronary arteries appears very similar to that of G-1 on CASC in that stimulation of BK_{Ca} channels is likely an important effector of

GPER-induced coronary artery relaxation. In contrast to these findings, however, are previous studies indicating that ICI182,780 can inhibit raloxifene-induced, endothelium-dependent relaxation of rabbit coronary arteries^[54], also attenuates raloxifene-induced NO production from human endothelial cells^[55]. Thus, it seems likely that endothelium-dependent effects of raloxifene are mediated primarily by classic ERs, whereas the direct effects on CASC are *via* GPER.

It is interesting that the diverse mechanisms mediating acute, non-genomic estrogen-induced coronary artery relaxation often converge upon a common cellular effector-the BK_{Ca} channel. Nearly 20 years ago we first demonstrated that E2 could activate this powerful hyperpolarizing mechanism in CASC^[11], and a number of studies have since confirmed and extended the role this protein plays in mediating acute estrogen signaling in coronary arteries. An exciting new development is that there is now increasing evidence that coronary artery relaxation induced by GPER activation appears to involve BK_{Ca} activity as well. In addition to E2, single-channel and whole-cell patch-clamp studies have demonstrated that agents known to stimulate GPER, *i.e.*, G-1^[46], raloxifene^[37], tamoxifen^[10], also exert significant stimulatory effects on BK_{Ca} channel activity in isolated CASC. Although not tested directly, it is also quite likely that the endothelium-dependent coronary artery relaxation effect of G-1^[36] may also indirectly open BK_{Ca} channels in CASC *via* release of NO^[10,11,56]. These cellular studies are bolstered by the fact that ibuprofen attenuates coronary relaxation induced by either G-1^[46] or raloxifene^[37]. At present, mechanisms coupling GPER activation to BK_{Ca} channel activity remain undefined; however, there is likely to be significant therapeutic potential here. For example, identification of the BK_{Ca} channel as a molecular effector of rapid estrogen signaling in CASC could lead to the development of new agents which could specifically target these proteins in coronary arteries to provide the beneficial vasodilatory effect of E2 without the substantial endocrine side effects of hormone treatment.

GPER AND CORONARY ARTERY CELL PROLIFERATION

In healthy arteries CASC retain a contractile phenotype and are localized in the medial layer; however, intimal injury (*e.g.*, atherosclerosis, angioplasty) causes CASC to dedifferentiate, lose their contractile phenotype, and proliferate^[57,58]. Dedifferentiated CASC can then migrate into the intimal region and contribute to the narrowing of the coronary artery lumen. Estrogen is known to inhibit injury-induced VSM proliferation^[46,59-64], but, interestingly, genetic deletion of classic ERs does not abolish this anti-proliferative effect of E2^[65,66]. Thus, it is likely that the protective, anti-proliferative effect of estrogens is due, at least in part, to activation of another ER-quite possibly GPER-in coronary arteries.

We recently reported that stimulation of GPER by G-1

inhibits proliferation of human and porcine CASC^[47]. In this study we found that 24-h exposure of primary porcine CASC to G-1 inhibited serum-induced cell growth *via* repression of cell cycle progression. Further, we found that G-1 completely inhibited CASC migration, and this inhibitory effect was attenuated by G-15. Similarly, Haas *et al.*^[50] found that G-1 decreased proliferation of human umbilical vein VSM. Further, VSM proliferation, assessed by measuring the media-to-lumen ratio, in murine resistance vessels was significantly increased in animals lacking the GPER gene^[67]. Thus, it is very likely that GPER helps maintain VSM cells in a differentiated, contractile phenotype, and may thereby help retard the development of atherosclerotic buildup in the vascular intima.

Estrogen is also known to regulate the proliferation of vascular endothelial cells, and, specifically, can influence endothelial cell growth and re-endothelialization^[68]. For example, direct delivery of E2 promotes reendothelialization and endothelial nitric oxide synthase expression in coronary arteries after damage due to coronary angioplasty^[69]. In addition to this protective effect that may promote healing of endothelial damage, there is also evidence that GPER may prevent excessive proliferation of endothelial cells. G-1 reduces proliferation, DNA synthesis, and number of microvascular endothelial cells^[70]. These studies suggest that an important role of GPER may be to provide an optimal balance for the effects of E2 on endothelial cell proliferation, and thereby prevent excessive endothelial cell proliferation; for instance, as occurs in tumor-associated angiogenesis.

The mechanism(s) of how GPER attenuates vascular cell growth remain to be elucidated, although several lines of evidence point to specific alterations in mitogenic signaling pathway such as extracellular signal-regulated protein kinases (ERKs) and protein kinase B (Akt). For example, E2 has been shown to phosphorylate ERK-1 and ERK-2 in breast cancer cells expressing GPER^[71], thus enhancing cell proliferation. In contrast, we recently reported that GPER activation decreases phosphorylation of ERK1/2 and Akt activity in human and porcine CASC^[47], thus suppressing proliferation. This decreased kinase activity was consistent with a similar inhibitory effect of GPER stimulation on ERK1/2 activity in breast cancer cells^[72]. Further, Gros *et al.*^[45] reported that E2 enhanced apoptosis in rat aortic VSM cells in which GPER was overexpressed, and did so in an ERK1/2-dependent manner. GPER overexpression altered downstream signaling from protein kinase A to a pertussis toxin-sensitive pathway which increased Akt phosphorylation and ERK1/2 activation, resulting in VSM cell apoptosis. In these VSM cells, G-1 stimulated ERK1/2 phosphorylation; however, other GPER agonists (*i.e.*, tamoxifen, ICI182,780) failed to do so. These studies indicate that E2 can induce cell apoptosis *via* GPER signaling; however, the signaling mechanisms underlying this effect are complicated and require further study. A summary of the currently known effects of GPER activation is presented in Table 1 and the proposed mechanisms mediating the effects of GPER activation in coronary arteries is sum-

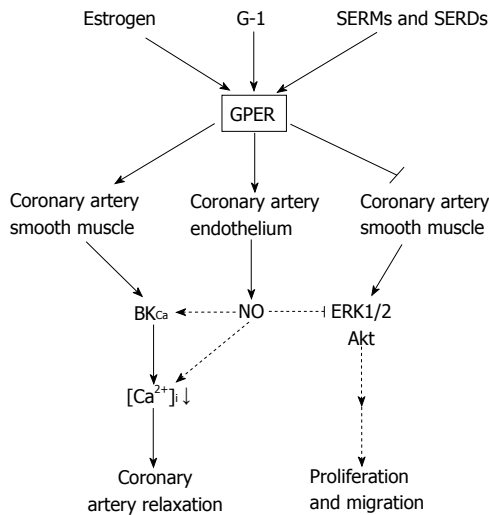


Figure 1 Summary of proposed mechanisms mediating the effects of G-protein-coupled estrogen receptor activation in coronary arteries. GPER is activated by 17 β -estradiol and the selective agonist, G-1. In addition, selective estrogen receptor modulators (e.g., raloxifene, tamoxifen) and selective estrogen receptor down regulators (e.g., ICI182,780) also appear to be agonists for GPER. GPER activation induces an endothelium-independent relaxation of coronary artery smooth muscle mediated by the large-conductance, calcium-activated potassium channel. In addition, GPER activation can stimulate release of NO from coronary endothelial cells to relax these arteries. Besides this vasodilatory effect, GPER activation can attenuate proliferation and migration of coronary artery smooth muscle cells by inhibiting signaling via the ERK1/2 and Akt pathways. GPER: G-protein-coupled estrogen receptor; SERMs: Selective estrogen receptor modulators; SERD: Estrogen receptor down regulator; ERK: Extracellular signal-regulated protein kinase.

marized in Figure 1.

GPER: A NOVEL THERAPEUTIC TARGET FOR CORONARY ARTERY DISEASE

The first rule of medical practice is to “do no harm”. Despite the considerable therapeutic potential of estrogen and estrogen-like compounds, fear of potentially dangerous ancillary effects continues to limit their usage. A primary obstacle to overcome is the pleiotropic effects of E2 on a wide diversity of tissues, most of which express one or more classes of ERs. For several years an important goal was to define the levels of ER expression in target tissues, and then deliver a receptor- or tissue-specific agonist that would produce the desired therapeutic response with limited side effects on non-target sites. However, because of the ubiquitous expression of ERs and the fact that multiple ERs are often expressed in the same cells, this goal has not been realized to any significant extent. With the more recent discovery of GPER, pursuit of this goal has been reinvigorated. In particular, the findings that GPER is highly expressed in both coronary endothelial cells and CASC has opened the search to understand the importance of this non-genomic estrogen signaling mechanism and explore pharmacological means whereby GPER activity could be modulated for therapeutic benefit. A summary of experimental evidence suggesting possible therapeutic benefits of GPER activa-

Table 1 Primary effects of G-protein-coupled estrogen receptor activation on coronary arteries

Effect	Species	Drug
Relaxation	Porcine	G-1 ^[36,46]
		ICI182,780 ^[35,36]
		Raloxifene ^[37]
		Tamoxifen ^[73]
		Raloxifene ^[54]
Endothelial NO production	Porcine	Tamoxifen ^[74]
		G-1 ^[36]
		Raloxifene ^[54]
CASC BKCa channel opening	Porcine	Tamoxifen ^[74]
		G-1 ^[46]
		Raloxifene ^[37]
Inhibition of CASC proliferation	Human	Tamoxifen ^[10]
	Porcine	G-1 ^[46]
	Human	G-1 ^[47]
Inhibition of CASC migration	Porcine	G-1 ^[47]

CASC: Coronary artery smooth muscle cells; BKCa: G-1 opens calcium-activated potassium; NO: Nitric oxide.

Table 2 Evidence for possible therapeutic effects of G-protein-coupled estrogen receptor agonists and selective estrogen receptor modulators

	Species	Drug
Coronary artery relaxation		
Endothelium-dependent, <i>in vitro</i>	Porcine	G-1 ^[36]
	Rabbit	Raloxifene ^[54]
Endothelium-independent, <i>in vitro</i>	Porcine	Tamoxifen ^[73,74]
		G-1 ^[46]
Reduced cardiac ischemic injury/infarct	Rat	Raloxifene ^[37]
		G-1 ^[75,76]
Reduced cerebral ischemic injury/infarct	Mice	G-1 ^[77]
Middle cerebral artery relaxation, <i>in vitro</i>	Rat	G-1 ^[78]
Systemic artery relaxation, <i>in vitro</i>	Mice	G-1 ^[40]
		G-1 ^[49]
		G-1 ^[50]
		G-1 ^[79]
	Rat	G-1 ^[51]
		G-1 ^[52]
Reduced systemic blood pressure, infusion	Human	G-1 ^[50]
		G-1 ^[49]
Inhibit VSM cell proliferation	Porcine	G-1 ^[50]
		G-1 ^[47]
Inhibit endothelial cell proliferation	Human	G-1 ^[50]
Prevents calcium-induced increases in plasma cholesterol	Mice	G-1 ^[70]
		G-1 ^[80]

VSM: Vascular smooth muscle.

tion is presented in Table 2.

CHD continues to be the greatest mortality risk factor in the developed world. Although our understanding of the causes of CHD continues to increase, therapeutic measures to prevent and treat this serious health problem have not improved dramatically over the past several decades. Invasive procedures such as bypass grafting or balloon angioplasty have been refined, but are still routinely practiced. Pharmacological measures (e.g., nitrates, calcium channel antagonists, beta blockers) can be effective at

treating the symptoms of CHD (*e.g.*, angina pectoris), but are seldom a viable long-term option, much less a cure. Ideally, what is needed is a widely-available therapeutic agent which might slow or reverse the progression of atherosclerosis, restore endothelial function, and induce coronary vasodilation in cases where blood flow was compromised significantly; and this agent would produce these beneficial cardiac effects with few side effects on other organs. In light of current research, it is possible that activation of GPER might be a promising new approach to achieving this desired therapeutic end.

Evidence is clear that activation of GPER produces an acute (*i.e.*, in minutes) dilation of coronary arteries due to relaxation of CSMC. This action appears to be both direct (acting on and relaxing CSMC) and indirect (*via* NO release from endothelial cells), and this dual action could prove to be very important as many CHD patients have dysfunctional or damaged coronary endothelium. Thus, stimulation of GPER has the potential to induce a direct coronary artery dilation, as well as lowering afterload due to its ability to decrease peripheral vascular resistance. As a consequence of GPER activation myocardial oxygen supply should increase with increased coronary blood flow as metabolic oxygen demand declines in face of lower peripheral vascular resistance. In addition, relaxation of venous smooth muscle could lower venous return and preload, thus further lowering myocardial oxygen demand. Thus, the vasodilatory potential of GPER activation could influence a number of favorable hemodynamic parameters to alleviate the pain and risk of CHD, and could be used acutely or prophylactically. In addition, there is similar evidence that GPER activation may also reduce the risk of ischemic stroke due to dilation of cerebral arteries^[77,78], and that GPER exerts a tonic suppression of arterial tone^[79].

Although we are only beginning to understand the mechanisms whereby GPER activation influences cell proliferation, there is accumulating evidence that GPER agonists exert an anti-proliferative and anti-migratory effect on CSMC-as it does for human urothelial cells^[81] and endothelial cells^[70]. Because CSMC dedifferentiation, proliferation, intimal migration, and secretion are important steps in the process of atherogenesis, these studies strongly suggest a potentially important protective effect of GPER activation on coronary atherosclerotic disease. Further, it appears that GPER activation can also help heal intimal damage and quite possibly help restore normal function to dysfunctional coronary endothelial cells-particularly because of its ability to enhance NO synthesis and release from endothelium. These intimal effects involving NO release would likely prevent coronary vasospasm and also help to further limit CSMC proliferation/migration, as well as attenuate the formation of coronary thrombi that could precipitate an acute ischemic attack or infarction. Although the potential effects of GPER stimulation of plasma lipoproteins are as yet unknown, a recent study has reported that GPER activation prevents increases in plasma total cholesterol levels in postmenopausal women taking calcium supplements^[80].

Thus, a new and promising effect of GPER activation may be outside the vascular system to help promote optimal cardiovascular health. Clearly then, there are potentially multiple sites of action for agents that would selectively stimulate GPER and produce beneficial effects on cardiovascular function-particularly treatment of CHD.

As always, potential side effects of GPER action must be considered. Initially, however, it could be predicted that GPER stimulation might produce significantly less risk of limiting side effects compared to E2 therapy or currently prescribed estrogenic agents (*e.g.*, breast or uterine cancer, venothromboembolism). For example, raloxifene has been demonstrated to lower overall risk of cardiovascular disease or breast cancer and strengthen bones in younger postmenopausal women^[82]; however, raloxifene does not lower blood pressure in these women, and its anti-estrogen side effects (*e.g.*, hot flashes, vaginal dryness) continue to limit its use somewhat. Tamoxifen has been widely employed as a treatment for estrogen-sensitive breast tumors. As a SERM, tamoxifen can increase bone density and produce beneficial changes in plasma lipids; however, its anti-estrogenic effects can increase the risk of uterine cancer and produce many negative symptoms of menopausal^[83]. It is likely that action on classic ERs (sometimes agonistic; other times antagonistic) mediates many of the undesirable side effects of SERM action.

At present, we are unaware of any reports from clinical trials evaluating the potential of G-1 (or another GPER agonist) as a therapeutic agent. As noted above, there appears to be great potential for GPER activation to enhance cardiovascular health. These effects, particularly those on coronary arteries, appear to be mediated almost exclusively *via* GPER with little or no concomitant activation of ER α or ER β . If so, then this more specific pharmacodynamic profile should do much to help limit the potential side effects of GPER activation on targets outside the cardiovascular system. A caveat, however, is that we are only beginning to understand the impact of GPER activation and its signaling mechanisms in a diversity of cell types. Thus, caution must be exercised in promoting GPER as a therapeutic target. Nonetheless, there is a substantial cautious optimism that pharmacological targeting of this novel non-genomic estrogen signaling mechanism may finally provide a means of producing the many beneficial effects of estrogen on the cardiovascular system while eliciting fewer side effects on the reproductive and other non-cardiovascular systems that continue to limit the use of other less specific estrogenic compounds.

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WJC 6th Anniversary Special Issues (2): Coronary artery disease

Effect of genetic factors on the association between coronary artery disease and PTPN22 polymorphism

Fulvia Gloria-Bottini, Patrizia Saccucci, Maria Banci, Paolo Nardi, Mattia Scognamiglio, Antonio Pellegrino, Egidio Bottini, Luigi Chiariello

Fulvia Gloria-Bottini, Patrizia Saccucci, Egidio Bottini, Department of Biomedicine and Prevention, School of Medicine, University of Rome Tor Vergata, 00133 Rome, Italy

Maria Banci, Department of Cardiology, Valmontone Hospital, 00038 Valmontone, Italy

Paolo Nardi, Mattia Scognamiglio, Antonio Pellegrino, Luigi Chiariello, Department of Cardiac Surgery, University of Rome Tor Vergata, School of Medicine, 00133 Rome, Italy

Author contributions: Gloria-Bottini F, Pellegrino A, Bottini E and Chiariello L contributed equally to the ideation of this research and wrote the paper; Saccucci P, Banci M, Nardi P and Scognamiglio M contributed to the data collection and statistical analysis; all authors contributed to the final revision of the paper.

Correspondence to: Fulvia Gloria-Bottini, MD, Department of Biomedicine and Prevention, School of Medicine, University of Rome Tor Vergata, Via Montpellier 1, 00133 Rome, Italy. gloria@med.uniroma2.it

Telephone: +39-6-30889514 Fax: +39-6-30889514

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without CAD. A similar pattern is observed in carriers of *Pro allele of p53 codon 72 with a higher proportion of *T allele carriers in non diabetic subjects with CAD as compared to other groups. A highly significant association between PTPN22 and CAD is observed in carriers of ADA₂ *2 allele with higher proportion of *T allele carriers in non diabetic subjects with CAD as compared to other group. There is a high significant correlation between the number of factors that contributes to increase the strength of association between PTPN22 *T and CAD and the proportion of *T carriers in CAD. ACP₁, p53 codon 72 and ADA are involved in immune reaction and give an important additive contribution to the strength of association between PTPN22 and CAD. This study stresses the importance of the simultaneous analysis of multiple genes functionally related to a specific disease: the approach may give important hints to understand multifactorial disorders.

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Key words: Coronary artery disease; PTPN22; Acid phosphatase locus 1; Adenosine deaminase 2; p53 codon 72

Core tip: Acid phosphatase locus 1, p53 codon 72 and adenosine deaminase have an important role in immune reactions and influence the strength of association between coronary artery disease (CAD) and PTPN22 an enzyme involved in autoimmunity. These results agree with multifactorial origin of CAD.

Abstract

PTPN22 has been previously found associated with coronary artery disease (CAD). In the present note we have studied the effect of p53 codon 72, acid phosphatase locus 1 (ACP₁) and adenosine deaminase (ADA) genetic polymorphism on the strength of association between PTPN22 and CAD. We have studied 133 non diabetic subjects with CAD, 122 non diabetic cardiovascular patients without CAD and 269 healthy blood donors. Informed written consent was obtained from all subjects and the study was approved by the Ethical Committee. A high significant association between PTPN22 and CAD is observed in carriers of *A allele of ACP₁ with a higher proportion of *T allele carriers in non diabetic subjects with CAD as compared to controls and to non diabetic subjects with cardiovascular disease

Gloria-Bottini F, Saccucci P, Banci M, Nardi P, Scognamiglio M, Pellegrino A, Bottini E, Chiariello L. Effect of genetic factors on the association between coronary artery disease and PTPN22 polymorphism. *World J Cardiol* 2014; 6(6): 376-380 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i6/376.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i6.376>

INTRODUCTION

PTPN22 gene encodes a protein tyrosine phosphatase expressed principally in lymphoid tissue and it is also named Lyp. PTPN22 protein is involved in the control of immune system activity. The gene shows a single nucleotide polymorphism C/T at +1858 resulting in the W620 variant that is associated to autoimmune diseases. We have previously found in non diabetic subjects an association of PTPN22 with coronary artery diseases (CAD)^[1] confirming the relationship observed by Pertovaara *et al*^[2] between PTPN22 and atherosclerosis.

p53 codon 72 shows a single nucleotide substitution resulting in the presence of either arginine or proline in the aminoacid sequence. Proline variant is a stronger transcriptional activator, while the arginine variant is a stronger apoptosis inducer. The impact of this polymorphism within the context of a living organism is poorly understood but several data indicate that it is involved in immunity and inflammation by regulating STAT 1 and pro-inflammatory cytokines^[3,4]. We have recently reported a statistically significant effect of this polymorphism on the association between PTPN22 and CAD in non diabetic subjects^[5].

Acid phosphatase locus 1 shows a genetic polymorphism that controls the synthesis of a low molecular weight protein tyrosine phosphatase. The protein is composed by two isoforms called F (fast) and S (slow). The polymorphism is due to the presence of three codominant alleles *A, *B and *C at an autosomic locus. The corresponding six genotypes show an increasing enzymatic activity in the order *A/*A < *A/*B < *B/*B ≤ *A/*C < *B/*C < *C/*C^[6]. The enzyme dephosphorylate a negative regulatory phosphorylation site of the ZAP70 tyrosine kinase in T cells that leads to increased activation of the kinase resulting in enhanced signaling from T-cell antigen receptor^[7]. This suggests that acid phosphatase locus 1 (ACP₁) could have an important role in immune functions. An association between ACP₁ and CAD has been reported^[8].

Adenosine deaminase (ADA) structural gene consists of 12 exons distributed in approximately 32 kb of DNA on chromosome 20^[9]. A number of differences among normal sequences have been found within exonic and intronic regions of the gene^[10]. The enzyme contributes to control the concentration of adenosine that in turn regulates T cell activation with important effects on immune reactions. As ectoenzyme ADA acts as a costimulatory molecule that facilitates specific signaling events in various cell types^[11].

We have studied three intragenic ADA polymorphisms (PCRP_s). The three PCRP_s spanning over about 28 kb have a known molecular basis and include the presence/absence of a Taq I site (ADA₁) (nt 4050-4053-exons 1), of Pst I site (ADA₂) (nt 19465-19470, intron 2) and a Mlu NI site (ADA₆) (nt 31230-31235, exon 6)^[10]. In non diabetic subjects with CAD a preliminary analysis of association of PTPN22 with the three ADA locus has revealed a statistically significant association with ADA₂

locus.

In the present note we have examined the cooperative effects of ACP₁, p53 codon 72 and ADA₂ genetic polymorphisms on the association of PTPN22 and CAD in non diabetic subjects.

EMPIRICAL STUDY

PTPN22 and ACP₁ genotype were determined in 133 non diabetic subjects admitted to hospital for CAD, in 122 non diabetic cardiovascular patients without CAD and in 269 healthy blood donors. PTPN22 and p53 codon 72 genotype were determined in 129 non diabetic subjects with CAD, in 117 non diabetic admitted for cardiovascular disease without CAD and in 256 healthy blood donors. PTPN22 and ADA₂ genotypes were determined in 132 non diabetic subjects with CAD, 121 non diabetic subjects with cardiovascular diseases without CAD and in 147 healthy blood donors. All the four polymorphisms, PTPN22, ACP₁, p53 codon 72 and ADA₂ were determined in 128 non diabetic subjects with CAD and in 117 non diabetic subjects admitted for cardiovascular diseases without CAD.

Informed written consent was obtained from all subjects to participate to this study that was approved by the Ethical Committee of the Hospital.

ACP₁, p53 codon 72, PTPN22 and ADA₂ genotypes was determined by DNA analysis. Technical details about the determination of the four polymorphisms have been described in previous papers^[12,13].

Statistical analysis was performed by using SPSS programs.

RESEARCH

Table 1 shows the proportion of *T allele of PTPN22 polymorphism in relation to the presence of *A allele of ACP₁ polymorphism in non diabetic subjects with CAD, in non diabetic cardiovascular patients with no CAD and in healthy subjects. A high significant association is observed in carriers of *A allele with a very high proportion of *T allele carriers in non diabetic subjects with CAD as compared to controls and to non diabetic subjects with cardiovascular diseases without CAD. Such association is not observed in subjects who do not carry the *A allele of ACP₁.

Table 2 shows the proportion of PTPN22 *T allele carriers in relation to the presence of *Pro allele of p53 codon 72 polymorphism in the three groups of subjects. A high significant association is observed in carriers of *Pro allele with a very high proportion of *T allele carriers in non diabetic subjects with CAD as compared to controls and to non diabetic subjects with cardiovascular diseases without CAD. Such association is not observed in subjects carrying the *Arg/*Arg genotype.

Table 3 shows the proportion of *T allele carriers in relation to the presence of the ADA₂ *2 allele of ADA₂ polymorphism in non diabetic subjects with CAD, in non diabetic subjects with cardiovascular diseases without

Table 1 Proportion of *T allele of PTPN22 in relation to the presence of *A allele of acid phosphatase locus 1 polymorphism

	Proportion of carriers of *T allele of PTPN22		Total of subjects, <i>n</i>
Non diabetic subjects with CAD			
Subjects carrying the *A allele	19.3%		62
Other ACP ₁ genotypes	7.0%		71
Non diabetic subjects with cardiovascular diseases without CAD			
Subjects carrying the *A allele	3.4%		59
Other ACP ₁ genotypes	6.3%		63
Blood donors			
Subjects carrying the *A allele	7.2%		138
Other ACP ₁ genotypes	4.6%		131
Statistical analysis	χ^2 test of independence		
	χ^2	df	P
Carriers of *A allele	10.598	2	0.005
Other ACP ₁ genotypes	0.998	2	0.742

CAD: Coronary artery disease; ACP₁: Acid phosphatase locus 1.**Table 2** Proportion of carriers of *T allele of PTPN22 in relation to the presence of the *Pro allele of p53 codon 72 polymorphism

	Proportion of carriers of *T allele of PTPN22		Total of subjects, <i>n</i>
Non diabetic subjects with CAD			
*Arg/*Arg genotype	7.6%		66
Carriers of *Pro allele	17.5%		63
Non diabetic subjects with cardiovascular diseases without CAD			
*Arg/*Arg genotype	9.2%		65
Carriers of *Pro allele	0.0%		52
Blood donors			
*Arg/*Arg genotype	7.2%		139
Carriers of *Pro allele	5.1%		117
Statistical analysis	χ^2 test of independence		
	χ^2	df	P
*Arg/*Arg genotype	1.212	2	0.545
Carriers of *Pro allele	11.248	2	0.004

CAD: Coronary artery disease. Adapted from reference [13].

Table 3 Proportion of carriers of *T allele of PTPN22 in relation to the presence of the adenosine deaminase locus 2 *2 allele of adenosine deaminase locus 2 polymorphism

	Proportion of carriers of *T allele of PTPN22		Total of subjects, <i>n</i>
Non diabetic subjects with CAD			
ADA ₂ *1/*1 genotype	8.3%		84
Carriers of ADA ₂ *2 allele	20.8%		48
Non diabetic subjects with cardiovascular diseases without CAD			
ADA ₂ *1/*1 genotype	6.7%		75
Carriers of ADA ₂ *2 allele	2.2%		46
Blood donors			
ADA ₂ *1/*1 genotype	4.5%		88
Carriers of ADA ₂ *2 allele	5.1%		59
Statistical analysis	χ^2 test of independence		
	χ^2	df	P
ADA ₂ *1/*1 genotype	1.024	2	0.599
Carriers of ADA ₂ *2 allele	11.747	2	0.003

CAD: Coronary artery disease; ADA₂: Adenosine deaminase locus 2.

CAD and in healthy blood donors. A high significant association is observed in carriers of ADA₂ *2 allele with a very high proportion of *T allele carriers in non diabetic subjects with CAD as compared to controls and to non diabetic subjects with cardiovascular diseases without CAD. Such association is not observed in subjects who

do not carry the ADA₂ *2 allele.

Figure 1 shows in non diabetic subjects with CAD the relationship between the number of factors (*i.e.*, *A allele of ACP₁, *Pro allele of p53 and ADA₂ *2 allele) which contributes to the increase of PTPN22 *T allele carriers, and the proportion of *T carriers. There is a highly

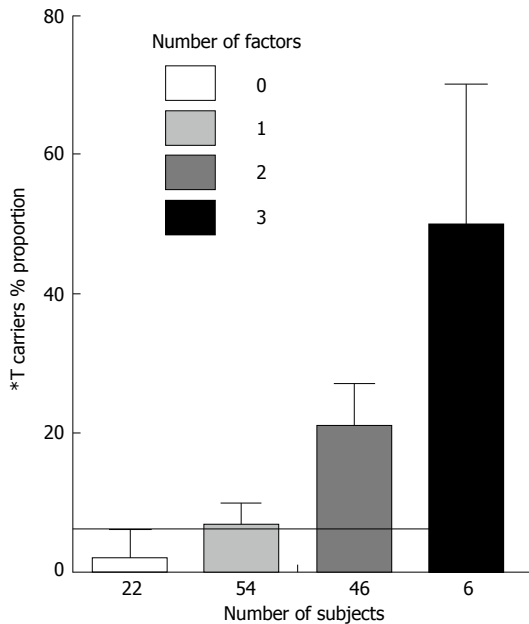


Figure 1 Twenty-two non diabetic subjects with coronary artery disease had no factor contributing to increase the proportion of *T carriers, 54 subjects had 1 factor, 46 had 2 factors and 6 had 3 factors.

significant linear correlation between the number of factors and the proportion of *T carriers (0.0004). The relationship is compatible with an exponential function $y = 5^x/100$ in which y = *T carriers proportion and x = the number of factors that influence the proportion of *T allele carriers.

Figure 2 shows a similar analysis in non diabetic subjects with cardiovascular disease without CAD. The relationship appears opposite to that observed in non diabetic subjects with CAD.

In non diabetic subjects with CAD we have examined the relationship of PTPN22 with sex, hypertension, magnetic resonance imaging, age and total cholesterol level. No statistical significant association has been observed.

CONCLUSION

The strength of association between PTPN22 and CAD in non diabetic subjects is dependent on other genetic variables. A similar phenomenon has been recently reported also in endometriosis, a disease in which immunological factors could have a important role^[14]. The data point to a multifactorial origin of CAD with a contribution of several genes involved in immune reactions.

It has been suggested that the increased susceptibility to autoimmune disorders observed in carriers of W620 variant of PTPN22 is due to failure to delete autoreactive T cells during intrathymic selection^[15,16]. The Proline variant with its stronger transcriptional activity could increase the production of autoreactive T cells enhancing the effect of W620 variant of PTPN22.

Low ACP1 activity decreasing ZAP70 activity, results in a weakening of T cell receptor signaling that may contribute with W620 variant to the failure to delete autore-

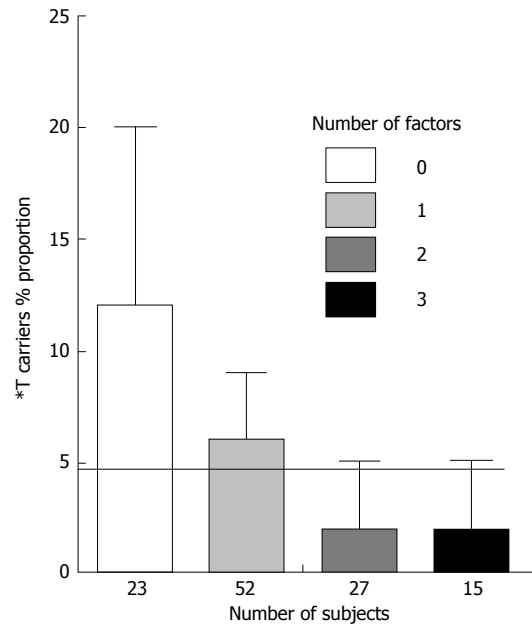


Figure 2 Twenty-three non diabetic subjects with cardiovascular diseases without coronary artery disease had no factor contributing to increase the proportion of *T carriers, 52 subjects had 1 factor, 27 had 2 factors and 15 had 3 factors.

active T cells during intrathymic induction.

ADA2 polymorphism could influence ADA activity and in turn the concentration of adenosine and T cell activity. The polymorphism also may have a role on ADA activity as ectoenzyme. The strength of the signal on lymphocyte would depend on the concentration of ecto-ADA available. Modulation of ecto-ADA function could influence the development and functionality of lymphoid tissue.

The simultaneous analysis of multiple genes functionally related to a specific disease would provide a productive approach to the analysis of multifactorial diseases. The mechanisms of the observed associations presented in this paper, however, remain to be elucidated.

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WJC 6th Anniversary Special Issues (2): Coronary artery disease

Coronary thrombus in patients undergoing primary PCI for STEMI: Prognostic significance and management

Sabine Vecchio, Elisabetta Varani, Tania Chechi, Marco Balducelli, Giuseppe Vecchi, Matteo Aquilina, Giulia Ricci Lucchi, Alessandro Dal Monte, Massimo Margheri

Sabine Vecchio, Elisabetta Varani, Marco Balducelli, Giuseppe Vecchi, Matteo Aquilina, Giulia Ricci Lucchi, Alessandro Dal Monte, Massimo Margheri, Division of Cardiology, Cardiac Catheterization Laboratory, Santa Maria Delle Croci Hospital, 48121 Ravenna, Italy

Tania Chechi, Division of Cardiology, Santa Maria Annunziata Hospital, 50100 Firenze, Italy

Author contributions: Vecchio S, Varani E, Chechi T and Balducelli M wrote the manuscript; Vecchi G, Aquilina M, Ricci Lucchi G, Dal Monte A and Margheri M revised the manuscript critically for important intellectual content.

Correspondence to: Sabine Vecchio, MD, Division of Cardiology, Cardiac Catheterization Laboratory, Santa Maria Delle Croci Hospital, Viale Randi n°5, 48121 Ravenna, Italy. sabinevecchio@gmail.com

Telephone: +39-0544-285745 Fax: +39-0544-285745

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Abstract

Acute ST-elevation myocardial infarction (STEMI) usually results from coronary atherosclerotic plaque disruption with superimposed thrombus formation. Detection of coronary thrombi is a poor prognostic indicator, which is mostly proportional to their size and composition. Particularly, intracoronary thrombi impair both epicardial blood flow and myocardial perfusion, by occluding major coronary arteries and causing distal embolization, respectively. Thus, although primary percutaneous coronary intervention is the preferred treatment strategy in STEMI setting, the associated use of adjunctive anti-thrombotic drugs and/or percutaneous thrombectomy is crucial to optimize therapy of STEMI patients, by improving either angiographical and clinical outcomes. This review article will focus on the prognostic significance of intracoronary thrombi and on current antithrombotic pharmacological and interventional strategies used in

the setting of STEMI to manage thrombotic lesions.

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Key words: ST-elevation myocardial infarction; Intracoronary thrombosis; Primary percutaneous coronary intervention; Antithrombotic therapies; Coronary thrombectomy

Core tip: Intracoronary thrombosis, is the basic pathophysiologic event in acute ST-elevation myocardial infarction (STEMI), and thrombi are very frequently detected in STEMI patients undergoing primary percutaneous coronary intervention (PPCI). Thrombus burden and components are important determinants of prognosis in STEMI, being well-known risk factors for long-term adverse cardiovascular events, distal embolization and stent thrombosis. As a result, percutaneous management of lesions with a consistent thrombotic burden is still challenging in the setting of PPCI for STEMI. Therefore, several pharmacological and interventional strategies, such as thrombectomy have been developed in order to improve PPCI's safety and efficacy, by reducing thrombus burden.

Vecchio S, Varani E, Chechi T, Balducelli M, Vecchi G, Aquilina M, Ricci Lucchi G, Dal Monte A, Margheri M. Coronary thrombus in patients undergoing primary PCI for STEMI: Prognostic significance and management. *World J Cardiol* 2014; 6(6): 381-392 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i6/381.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i6.381>

INTRODUCTION

Intracoronary thrombosis, subsequent to plaque rupture and causing partial or complete occlusion of a coronary

artery, is the basic pathophysiologic event in acute ST-elevation myocardial infarction (STEMI)^[1]. Actually, although angiography seems to underestimate the presence of thrombi, they are very frequently detected in STEMI patients undergoing primary percutaneous coronary intervention (PPCI) and tend to be larger than in non ST-elevation acute coronary syndromes (ACS). Sianos *et al*^[2] reported that up to 91.6% of STEMI patients undergoing PPCI showed intracoronary thrombosis at angiography.

Intracoronary thrombus burden and components are important determinants of prognosis in STEMI, being well-known risk factors for long-term adverse cardiovascular events, distal embolization and stent thrombosis^[2-8]. As a result, percutaneous management of lesions with a consistent thrombotic burden is still challenging in the setting of PPCI for STEMI. Therefore, several pharmacological and interventional strategies, such as thrombectomy, aiming at reducing thrombus burden, have been developed in order to improve PPCI's safety and efficacy, patients' survival and their quality of life.

CORONARY THROMBOSIS IN STEMI PATIENTS

Atherosclerotic plaque rupture or erosion are usually followed by hemorrhage into the plaque, luminal thrombosis, and vasospasm, which may cause sudden, partial or total, flow obstruction, and hence the onset of ischemic symptoms in the setting of STEMI^[1,9,10]. Inflammation and increased oxidative stress seem to play an important role in the pathogenesis of plaque instability^[11-13], while the clinical manifestation of an acute thrombotic event is determined by the balance between the propensity for thrombus formation, proportional to the kind and extent of exposed plaque components and to the local flow disturbances, and the efficacy of endogenous thrombolytic processes^[14]. However, plaque disruption and thrombosis do not always coincide with the onset of symptoms^[15,16]. Actually, post-mortem investigation and, more recently, histological studies of *in vivo*-derived thrombectomy specimens of STEMI patients, revealed that approximately 50% of the aspirated thrombi were days to even weeks old, which further suggests that thrombus formation starts at a variable time before symptoms onset^[17,18].

Pathological analyses revealed that coronary thrombi consist of platelets, erythrocytes and fibrin, and often contain atherosclerotic inflammatory cells^[19,20]. Initially, at the site of plaque disruption, platelets aggregate forming a platelet-rich thrombus which begins to protrude into the lumen. Then, the thrombus grows in association with the formation of a fibrin network entrapping a lot of erythrocytes and inflammatory cells, and forming an erythrocyte-rich thrombus^[19-21], which can partially or totally occlude the vessel.

ANGIOGRAPHIC CORONARY THROMBI: DEFINITION AND CLASSIFICATION

Angiography seems to underestimate the presence of

thrombi. Nevertheless, intracoronary thrombi are angiographically defined as the presence of a filling defect with either a total occlusion with convex, irregular, or hazy distal margins and post injection contrast retention or staining, or a partial occlusion circumferentially outlined by contrast medium^[22].

When angiographically detected, the thrombus burden can be classified according to the thrombolysis in myocardial infarction (TIMI) thrombus grade (TG)^[23]. TIMI TG 0 corresponds to no angiographic evidence of thrombus; in TIMI TG 1, angiographic characteristics suggestive of thrombus are detected (*i.e.*, reduced contrast density, haziness, irregular lesion contour or a smooth convex meniscus at the site of total occlusion suggestive but not diagnostic of thrombus); in TG 2, there is definite thrombus, with greatest dimensions $\leq 1/2$ the vessel diameter; in TG 3, there is definite thrombus but with greatest linear dimension $> 1/2$ but < 2 times the vessel diameter; in TG 4, there is definite thrombus, with the largest dimension ≥ 2 vessel diameter; and in TIMI TG 5, there is total occlusion and the size of thrombus cannot be assessed.

In STEMI setting, there is a high incidence of total coronary occlusion, thus, as was shown by Sianos *et al*^[2], the prevalence of TG 5 and unknown thrombus size is almost 60% of the patients. Therefore, a modified TG classification was recently suggested by Sianos *et al*^[2], where, grade 5 lesions are reclassified into one of the other TIMI grade categories, after flow achievement with either guidewire crossing or a small (diameter 1.5 mm) deflated balloon passage or dilation. According to this new classification, most lesions (99%) can be classified. Particularly, TIMI TG 0-3 are defined as small thrombus burden (STB), while TIMI TG 4 is defined as large thrombus burden (LTB).

PROGNOSTIC SIGNIFICANCE OF ANGIOGRAPHICALLY DETECTED CORONARY THROMBI

Angiographic detection of coronary thrombi in the setting of PPCI for STEMI is a well known negative prognostic factor, associated with a higher incidence of in-hospital and long-term adverse cardiac events^[2,6,24,25]. Actually, intracoronary thrombi can impair both epicardial and myocardial perfusion, by spontaneous or PPCI-induced occlusion of an epicardial vessel or its branches, or distal embolization of plaque and thrombotic components. Data derived from PPCI for STEMI studies, showed that PPCI resulted in about 6% to 18% distal embolization rate^[3,4,25-28]. Moreover, patients with distal embolization, compared to those without, showed lower procedural success rates with higher slow/no-reflow rates, lower left ventricular ejection fraction (LVEF), larger enzymatic infarctions, with increased in-hospital and late mortality rates^[25,29].

Size and thrombus composition are the major predictors of distal embolization, as well as slow TIMI flow

grade before PCI, long target lesion and large vessel diameter^[3,4,30]. A LTB and a high plaque burden were shown to be independent predictors of distal embolization^[2,5,7,29], and correlated with worse final TIMI flow/myocardial blush grades, as well as 2-year mortality and major adverse cardiac event (MACE) rates^[2]. In STEMI setting, thrombus burden is higher than in the other types of ACS. Particularly, a significant association between TG and vessel size has been reported (*i.e.*, large right coronary arteries, aneurismatic coronary arteries and aged degenerated saphenous vein grafts). Moreover, some clinical scenarios, such as STEMI occurring for stent thrombosis (ST), are associated with the presence of a LTB. Actually, Chechi *et al.*^[31] reported a significantly higher incidence of LTB (TG ≥ 3) in patients with STEMI due to ST, compared to those with STEMI due to *de novo* coronary thrombosis.

Recently, the development of thrombectomy and distal protection devices has enabled the evaluation of ante-mortem coronary thrombi, thus facilitating analysis of thrombi components, given that previous autopsy studies were unable to differentiate coronary thrombi responsible for STEMI from post-mortem clots. However, even for in vivo-derived thrombectomy thrombi, a sampling bias must be considered, related to the inability to determine whether retrieval of the thrombus was complete and which part of the thrombus has been extracted, and to the potential distortion of the samples that might have occurred during aspiration through a catheter lumen. Nevertheless, recent studies have demonstrated that erythrocyte-rich component in aspirated coronary thrombi is closely associated with thrombus size that increases the incidence of distal embolization during PPCI in STEMI patients^[4,32]. Actually, data on aspirated thrombi from 164 STEMI patients within 12 h from symptoms onset, revealed that thrombi from patients with distal embolization had a greater erythrocyte-positive area and more myeloperoxidase (MPO)-positive cells than those from patients without distal embolization, and that thrombus size was positively correlated with the erythrocyte component and the numbers of MPO-positive cells^[32]. These results reflect the above mentioned mechanism of thrombosis whereby the thrombus, initially platelet-rich, becomes erythrocyte-rich with inflammatory cells entrapped during thrombus growth^[21,33,34]. Moreover, MPO-positive cells, constituted by neutrophils and only occasionally by macrophages^[35], and erythrocyte-rich thrombi were shown to be associated with impaired coronary microcirculation, as assessed by ST-segment resolution and myocardial blush grade after PPCI in STEMI patients^[36,37]. Finally, independently from the histopathology of aspirated thrombi, patients with fresh thrombus tended to have better ST-segment resolution than patients with older thrombus^[38].

Prediction of thrombus burden and composition, as well as plaque volume and composition, before the procedure in patients with STEMI undergoing PPCI, may contribute to optimize percutaneous treatment of these highly thrombotic lesions, guiding utilization of pharma-

cological agents or interventional strategies, in order to reduce thrombus burden and improve both epicardial and myocardial perfusion. Grade III ischemia on electrocardiogram, defined as distortion of the terminal portion of the QRS complex, and red cell distribution width (RDW), a marker of variation in the size of circulating red cells routinely reported as a part of blood count analysis, were shown to be independent predictors of coronary thrombus burden in STEMI patients undergoing PPCI, and to be associated with angiographic no-reflow and impaired epicardial and myocardial perfusion^[39-41]. Probably, also the evaluation of thrombus burden using, not only coronary angiography, but also intravascular imaging modalities, such as ultrasound, optical coherence tomography or virtual histology, may provide important informations about the amount and composition of coronary thrombi, thus facilitating the choice of treatment strategies.

PHARMACOLOGIC AND PERCUTANEOUS INTERVENTIONAL MANAGEMENT OF CORONARY THROMBI

PPCI is the preferred treatment option, compared to thrombolytic therapy, in STEMI patients, being effective in obtaining patency of the infarct-related artery (IRA)^[42], and resulting in smaller infarcts, less acute and long-term clinical events, including recurrent myocardial infarction and death^[43,44]. However, a substantial number of STEMI patients, up to 40%, treated with PPCI shows poor procedural outcomes^[25], above all because of the presence of intracoronary thrombi that can lead to micro and macro distal embolization, thus reducing the benefits of PCI^[25]. Actually, although PPCI effectively restores flow in the IRA, myocardial perfusion often remains sub-optimal, with persistent ST-segment elevation, abnormal myocardial blush grade and abnormal TIMI frame count, due to microvascular obstruction, mostly attributed to distal embolization^[45]. As a result, management of lesions with a consistent thrombotic burden is still challenging during PPCI for STEMI. This has led to the employment and development of drugs and adjunctive percutaneous devices, aiming at reducing distal embolization and therefore improve myocardial perfusion. Particular subgroups of STEMI patients may benefit more from these adjunctive pharmacological and interventional strategies; these include patients with large anterior myocardial infarction, LTB, residual thrombus, side-branch involvement, and those with slow or no-reflow. Finally, attention must be paid on stenting strategies in order to further reduce PCI complications.

Pharmacologic agents

Several pharmacologic agents, delivered intravenously or *via* the intracoronary route, can be used in the catheterization laboratory, to manage lesions with consistent thrombus burden during PPCI for STEMI. When possible, STEMI patients undergoing PPCI should receive dual

antiplatelet therapy (aspirin plus one of the ADP receptor blockers) and one parenteral anticoagulant. Moreover glycoprotein II b/IIIa inhibitors (GPI) and vasodilators drugs may be useful to manage lesions with consistent thrombus burden and to improve epicardial and myocardial perfusion. Particularly, these pharmacological measures are useful in the presence of slow or no-reflow, which is related to a combination of distal embolization of plaque debris and thrombus, vasoconstriction and reperfusion injury^[25].

Anticoagulants

Anticoagulant options for PPCI include unfractionated heparin (UFH), enoxaparin and bivalirudin. UFH titrated to an appropriate activated clotting time is a familiar and well-tested strategy for anticoagulant therapy in the setting of PPCI^[46,47], compared to enoxaparin which has been studied less extensively in this setting. Moreover, the ATOLL trial comparing intravenous enoxaparin with UFH for PPCI failed to meet its primary composite endpoint (30-d death, complication of myocardial infarction, procedural failure and major bleeding)^[48]. Thus, European guidelines recommend UFH in Class I, level of evidence C, while enoxaparin has an indication of Class II b, level of evidence B^[42]. However, European guidelines stated that enoxaparin should be preferred over UFH^[42], based on the considerable clinical experience with enoxaparin in other PCI settings^[42] and on considerations derived from the ATOLL trial^[48]. Particularly, although the primary endpoint was not reached, there were reductions in the composite main secondary endpoint of death, recurrent myocardial infarction or ACS or urgent revascularization, and in other secondary composite endpoints, such as death, or resuscitated cardiac arrest and death, or complication of myocardial infarction, and there was no indication of increased bleeding from use of enoxaparin over UFH^[48]. Moreover, a recent meta-analysis of 23 trials, including 30966 patients undergoing PCI (33.1% PPCI for STEMI, 28.2% rescue PCI, and 38.7% with non ST-elevation ACS or stable patients), showed that enoxaparin was associated with a significant relative and absolute risk reduction of mortality, along with a significant reduction of major bleeding, especially in patients treated with PPCI for STEMI^[49]. Bivalirudin is a direct thrombin inhibitor. In the HORIZONS-AMI trial^[50], reporting on 3602 STEMI patients randomized to UFH plus a GPI or to bivalirudin alone, the later showed lower major bleeding rates at 30-d, 1 and 3 years^[50-52], with significantly lower rates of death from cardiac causes and all causes^[50]. Conversely, the use of bivalirudin was associated with an initial increase in ST, which disappeared after 30 d^[50]. Based on these data, European guidelines recommend the use of bivalirudin, over UFH, in STEMI patients, with a Class I indication, level of evidence B, with use of GPI restricted only to bailout^[42].

GPIs

GPIs (abciximab, tirofiban and eptifibatide) inhibit final

common pathway of aggregation process by preventing fibrinogen from binding to activated platelets and forming white thrombus. All GPI agents have been found to achieve their benefits by reducing the clot burden at the epicardial coronary level, by improving microvascular flow and reducing no-reflow and infarct size, and thus by improving short- and long-term outcomes^[53,54]. Although, GPIs are frequently administered to ACS patients undergoing PCI, a strategy supported by several randomized clinical trials, their role in STEMI patients, treated with PPCI and dual antiplatelet therapy, has been conflicting, especially because of bleeding concerns^[50,55-57]. The most profound evidence has been found for abciximab, which remains the drug of choice in PPCI, in combination with heparin^[58,59]. The recent 2013 ACC/AHA guidelines^[47] have given the routine use of upstream GPIs in STEMI patients undergoing PPCI, a Class II b recommendation. However, upstream administration of GPIs, may be considered among high-risk patients within the first 4 h from symptoms onset, when the larger amount of myocardium at risk and viable myocardium may justify this approach^[60]. Actually, the On-TIME 2 trial showed that upstream administration of GPI was associated with a higher rate of an open artery and a lower initial thrombus burden, with these benefits restricted only to early presenters (< 76 min)^[61]. Therefore, GPIs, as stated by European guidelines^[42], should be considered only for bailout therapy (Class II a, C) if there is evidence of LTB, slow or no-reflow or a thrombotic complication, or could be administered upstream only in high-risk patients undergoing transfer for PPCI (Class II b, B). Generally, GPIs are administered intravenously. Recently, intracoronary bolus of abciximab has been tested, with the rationale that intracoronary drug concentration may increase drug efficacy, and that the continuous intravenous infusion may not be beneficial to further improve outcomes, but may increase the risk of bleeding, especially in the contemporary era of PPCI, in which more potent ADP receptor blockers and thrombus aspiration are available for most of the STEMI patients. However, to achieve these favorable effects, it is advisable to administer intracoronary abciximab bolus after thrombus penetration by the PCI guidewire, and when the risk of bleeding is an issue, intracoronary bolus of GPI and no infusion strategy may be useful. Some small studies showed infarct size reduction, decrease in microvascular obstruction, improvement in the LVEF, and improvement in myocardial blush, but no significant difference in the clinical outcomes with intracoronary bolus administration of abciximab, with and without subsequent infusion^[62-65]. However, meta-analyses published recently, demonstrated not only a favorable effect of intracoronary bolus on TIMI flow, but also on target vessel revascularization and short-term mortality after PCI with no increase of bleeding complications^[66,67]. In summary, the role of intracoronary bolus of GPIs still need to be established by randomized trials comparing intravenous and intracoronary GPIs administration, with and without subsequent infusion, in combination with

modern PPCI strategies.

Vasodilators

Vasodilators that have been used in PPCI setting include nitroprusside, adenosine and diltiazem or verapamil. When used, they are administered intracoronary, in order to achieve a higher local concentration. Thus, they can be delivered directly through the guiding catheter, or *via* a distal over-the-wire balloon, infusion catheters or infusion balloons^[68].

Adenosine is considered a cardio-protective agent, because it antagonizes many of the factors implicated in the reperfusion injury, and has been shown to reduce post-ischemic ventricular dysfunction and myocyte necrosis and apoptosis. Moreover, several studies showed beneficial effects on coronary flow^[69,70]. Compared to the other drugs, adenosine has the advantage to have a very short half-life, and therefore, adverse effects are rapidly resolved.

Nitroprusside is a direct donor of nitric oxide, functioning as a potent venous and arterial vasodilator. Selective intracoronary nitroprusside administration is safe, generally well-tolerated, and provides stimulus to promote vascular dilation and improve tissue perfusion, especially in patients who develop slow or no-reflow after PCI. Moreover, if administered before balloon or stenting angioplasty, intracoronary nitroprusside, as well as adenosine, may decrease rates of no-reflow, increase myocardial blush scores, and shorten procedural times. In cases of impaired flow during PCI, combination therapy of adenosine and nitroprusside has been shown to be safe and provides better improvement in coronary flow and MACE, as compared with adenosine alone^[68].

Small trials suggest that there may be a role for prophylactic use of intracoronary calcium channel blockers, especially verapamil, because they seem to prevent no-reflow in some patients by reversing the calcium-mediated distal microvascular spasm^[71,72].

Although the benefit of intracoronary delivery of adjunctive pharmacologic agents such as calcium channel blockers, adenosine and nitroprusside is limited to small studies showing reduction of embolization rates and not clinical outcomes, they are still useful in the catheterization laboratory.

Percutaneous devices

The rationale for thrombectomy and embolic protection devices use is the reduction of the incidence of distal embolization, and improvement of myocardial perfusion and clinical outcomes. Particularly, thrombectomy devices aim at reducing thrombus burden, while embolic protection devices aim at capturing the debris liberated during PCI.

Thrombectomy devices

In the last years thrombectomy has emerged as a useful tool to reduce thrombus burden and thus distal embolization, further enhancing benefits of PPCI. Various throm-

bectomy devices have been developed allowing manual or mechanical removal of intracoronary thrombi. All thrombectomy devices have shown benefits compared with conventional PPCI, when surrogate endpoints, such as angiographic flow assessment, LVEF assessment, infarct size reduction by perfusion imaging, enzymatic analysis and ST-segment resolution were used^[26,29,73-79]. To date evidences about hard endpoints from randomized controlled trials, comparing manual and mechanical thrombectomy, are limited and even conflicting.

The REMEDIA trial, comparing thrombus aspiration with the Diver CE (Invatec, Brescia, Italy) before PCI *vs* conventional PPCI^[73], showed no difference in clinical outcomes or peak creatine kinase, muscle and brain (CK-MB) elevation, but a significant improvement in perfusion grades and in ST-segment resolution. The EXPIRA trial, evaluating the Export catheter (Medtronic, Inc, Minneapolis, MN) in PPCI, demonstrated improvement in surrogate markers, including myocardial blush grade and ST-segment resolution^[78]. The TAPAS trial is the largest randomized trial to date evaluating thrombus aspiration in PPCI for STEMI^[26]. It randomized 1071 patients and demonstrated effective manual thrombus aspiration in 73% in the treatment group. There was a trend toward less MACE at 30 d.

Recently, a direct and adjusted indirect meta-analysis of studies on manual and mechanical thrombectomy in PPCI for STEMI has been published^[80]. The direct meta-analysis showed comparable rates of survival, re-infarction and procedural outcomes between the two groups, even though these results are limited in sample size. On the contrary, the indirect meta-analysis showed a superior reduction in mortality with manual compared to mechanical thrombectomy. When trials, such as TAPAS and AIMI, with low percentage of patients with intracoronary thrombus (< 50%) at baseline, were excluded from the analysis, the two strategies were comparable in survival, but mechanical thrombectomy was associated with a significant reduction in re-infarction and stroke^[80]. This report lends support to mechanical thrombectomy, which until now was looked upon with suspicion. Actually, despite more bulky and complex to use, mechanical thrombectomy devices may provide more consistent advantages in removal thrombus, because of their intrinsic properties. To date, the negative results associated with the use of mechanical thrombectomy devices, are mostly driven by the results of the AIMI trial^[81], reporting on 480 patients randomized to AngioJet rheolytic thrombectomy (RT) and standard PPCI. In this study, the AngioJet RT group reported a higher final infarct size, a lower final TIMI flow grade 3 and a higher 30-d MACE rate. It has been speculated that the higher mortality observed in these patients may be related to a very low (and unexpected) mortality of patients treated only by PPCI (0.8% *vs* 4.6%; $P = 0.02$)^[81]. Moreover, both operator experience and the technique used, might have influenced mortality in patients treated with AngioJet RT. Actually, in the AIMI trial enrolling centers were low-volume centers without

extensive AngioJet experience, as resulted from the high rate of coronary perforation. Furthermore, a retrograde thrombectomy technique was used without activation of the device prior to crossing the lesion, which might have promoted distal embolization. Finally, angiographic evidence of thrombus was absent in a large percentage of both groups^[81]. Conversely, the recently published JET-STENT trial, evaluating 501 patients with LTB (thrombus grade ≥ 3) in large vessels (≥ 2.5 mm), randomized to AngioJet RT prior to direct stent *vs* direct stent alone, reported that patients treated with AngioJet showed a better myocardial reperfusion, with a higher rate of early ST-segment resolution ($P = 0.043$), without any significant differences in secondary surrogate endpoints, such as infarct size at 1-mo scintigraphy, post-procedural TIMI flow and corrected TIMI frame count. On the contrary, the rate of MACE (*i.e.*, death, myocardial infarction, repeated revascularization and stroke) was significantly lower in patients treated with AngioJet either at 1-mo ($P = 0.043$), or 6-mo ($P = 0.011$) or 12-mo ($P = 0.036$) follow-up, primarily driven by a lower incidence of death and time to target vessel revascularization. This was attributed to better myocardial perfusion and to better stent length and diameter assessment following RT^[82].

Therefore, current evidences support the routine use of manual thrombectomy devices in PPCI, and consequently, manual thrombectomy received a Class IIa indication in PPCI in the recent ESC guidelines^[42]. However, when LTB is present, especially in large vessels and when experienced operators are available, mechanical thrombectomy with AngioJet system should be considered. Particularly, AngioJet may be very useful in patients with STEMI due to stent thrombosis, in which the thrombotic burden seems to be huge. A study published by Chechi *et al*^[31] showed that thrombus grade ≥ 3 was observed in all patients with STEMI due to stent thrombosis, compared to 93.9% of patients with STEMI and de-novo coronary thrombosis ($P = 0.01$). The OPTIMIST study, in which 110 patients with stent thrombosis treated by PCI have been evaluated, showed that a sub-optimal coronary reperfusion was related to a worse outcome, even though GPIs, intra-aortic balloon pump and mechanical thrombectomy devices were used. In this study, mechanical thrombectomy devices were under-used: only 30% of patients have been treated with these devices, and among them few have been treated with AngioJet^[83]. Patients treated with mechanical thrombectomy showed a better coronary reperfusion, compared to patients in which mechanical thrombectomy devices were not used^[83].

Embolic protection devices

Embolic protection devices (EPD) can be divided into proximal and distal devices. Distal EPDs consist in filter-wire or occlusive distal balloon systems, while the principal proximal EPD is represented by an occlusive proximal balloon system (*i.e.*, Proxis system). Few data are available on proximal EPDs, while most of the data regard distal EPDs. Distal EPDs were first used to protect from em-

bolization associated with PCI in diseased saphenous vein grafts, then after they were applied in PPCI setting for STEMI to protect myocardium during intervention on highly thrombotic lesions in native vessels.

The EMERALD trial demonstrated no significant improvements in the primary end points of myocardial reperfusion or infarct size with the use of the distal balloon occlusion and aspiration system, GuardWire, despite the removal of visible debris in a high proportion of patients (73%)^[84]. The DEDICATION trial, evaluating patients randomized to distal protection using a filter wire (Filter-Wire-EZ), or a SpiderFX protection device, *vs* standard PPCI without distal protection, showed no significant difference in the primary endpoint of ST-segment resolution or in cardiac biomarker elevation or left ventricular wall motion index, and found a higher MACCE rate with distal protection^[85]. Thus, although, distal EPDs showed favorable clinical benefits during PCI in saphenous vein grafts, the results in PPCI setting for native vessel were not so good. Resuming, no differences were reported on ST-segment resolution, infarct size and MACE rates with distal EPDs compared to standard PPCI^[84-86]. These data were confirmed by the meta-analysis by Kunadian *et al*^[87], where the use of distal EPDs resulted in no decrease of early mortality or recurrent myocardial infarction rate. Probably, the absence of benefits with the use of distal EPDs could be explained by the fact that such devices can themselves induce distal embolization when crossing highly thrombotic lesions and may not be completely effective in preventing all debris from embolizing. Theoretically, compared to distal EPDs, proximal ones offer the benefit of embolic protection without crossing the thrombus, therefore avoiding added distal embolization, while allowing effective thrombus removal. Conversely, proximal EPDs, such as the Proxis device, have several technical limitations contraindicating their use during PPCI (*i.e.*, the presence of a stenosis within 15 mm of the ostium or IRA proximal segment diameter < 2.5 or > 4.5 mm, contraindicate the use of Proxis system), and therefore making results on their use inconclusive. In the setting of STEMI, use of the Proxis device demonstrated an initial benefit in ST-resolution; however, this benefit was not maintained over time with a late catch-up in the control group.

Based on the above data, European guidelines did not recommend routine use of distal EPDs^[42].

Stenting strategies

PCI strategies, including selection of vascular access, timing of stenting, sizing and type of stent, are crucial to further improve angiographic and clinical outcomes during PPCI for STEMI, along with the use of adjunctive pharmacologic drugs and thrombectomy devices.

Compared to elective procedure, PPCI is associated with a higher rate of bleeding, because of the need for potent antithrombotic and antiplatelet agents, mostly related to the arterial puncture site. Radial approach has been shown to reduce the incidence of acute bleeding

events, both in ACS and STEMI patients, especially when operators are skilled with this arterial approach^[42].

The presence of a LTB in STEMI setting, may affect stent apposition, correct stent sizing and final TIMI flow, all of which are predictors of acute ST. Thus, the best approach to stenting in PPCI seems to be thrombus guided, as reported in the SINCERE database^[88]. Based on this strategy, if the extent of thrombus is small (TG 0-1), direct stenting may be sufficient. Conversely, if more significant thrombus burden is present (TG 2-3), initial aspiration with a manual device is usually prudent, by decreasing distal embolization and no-reflow, and facilitating subsequent stenting. If thrombus burden is unchanged after 2 passes, it is advisable to switch to a more aggressive thrombectomy device, such as the AngioJet system. If a very LTB is present (TG 4-5), manual thrombectomy may be insufficient and AngioJet RT may be warranted^[88]. Actually, a LTB has been related to a very high rate of ST (2); moreover, if not removed, thrombus compression or displacement by the stent struts may cause distal embolization and no-reflow in the acute phase during PPCI, and in the long-term, with abluminal thrombus resolution, may cause late stent malapposition, thus increasing the risk of late ST. Therefore, a strategy of delayed stent implantation (DSI) after thrombus removal, compared to immediate stent implantation (ISI), appears attractive. To date, only few and small studies have been published comparing these two strategies^[89-91], but they all showed that DSI is associated with better microvascular perfusion, less frequent distal embolization and no-reflow, compared with ISI. Certainly, in STEMI setting with LTB, DSI has to be weighed against the potential risk of recurrent ischemia and bleeding episodes during the waiting period before PCI. On the other hand, DSI could allow to perform PCI after full antithrombotic preparation, enhancing clot lysis and thrombus dissolution, and after enough time to “cool off” the culprit lesion, thus becoming more stable with a reduced incidence of adverse angiographic events.

When a stenting strategy is applied, selection of the appropriate stent diameter may be of particular importance during PPCI, since stent undersizing is one of the most powerful predictor of ST among non-elective PCI^[92]. Actually, the reference vessel diameter of the IRA may be difficult to accurately assess during PPCI, because of thrombus burden, catecholamine stimulation and inflammatory substances, that can contribute to general and localized vasoconstriction^[93]. Therefore, intracoronary administration of nitrates is recommended before starting the coronary angiographic sequence used for stent size selection^[42].

Drug-eluting stents (DES) can be implanted during PPCI for STEMI, with a reduced risk of repeated target vessel revascularization, compared with bare-metal stents (BMS)^[94]. There have been concerns about increased risks of very late ST and reinfarction with DES, compared with BMS^[94]. However, use of DES has not been associated with an increased risk of death, myocardial infar-

tion or ST on long-term follow-up^[52]. Moreover, newer generations of DES seem to provide improved clinical outcomes following PPCI, with a reduced incidence of ST. The often spastic reaction of the IRA and the presence of LTB, may be the rationale for implanting stents with progressive self-apposing after their implantation. Interim results of the APPPOSITION III trial, using self-expanding BMS, are promising with a lower 30-d MACE rate. Moreover, when a LTB is present, the use of special mesh-covered stents can be useful in managing thrombi and preventing distal embolization. There are some small studies reporting on the use of the MGuard stent in STEMI setting, documented promising surrogate results, such as a better ST-resolution and a higher post-procedural TIMI 3 flow rate, when compared to standard types of stents^[95-98].

CONCLUSION

Since detection of intracoronary thrombi is associated with distal embolization, myocardial damage and poor clinical outcomes, several pharmacologic agents and interventional adjunctive techniques need to be taken in consideration during PPCI for STEMI, as well as a correct stenting strategy. The treatment during PPCI needs to be modified with respect to the risk profile, thrombotic burden, availability of medical resources and operators' experience.

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Use of intravascular imaging in managing coronary artery disease

Sanda Jegere, Inga Narbute, Andrejs Erglis

Sanda Jegere, Inga Narbute, Andrejs Erglis, Latvian Centre of Cardiology, Pauls Stradins Clinical University Hospital, Riga, LV1002, Latvia

Sanda Jegere, Inga Narbute, Andrejs Erglis, Institute of Cardiology, University of Latvia, Riga, LV1002, Latvia

Author contributions: Jegere S, Narbute I and Erglis A contributed equally to this work; they analyzed the data, and wrote the manuscript.

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Correspondence to: Andrejs Erglis, MD, PhD, Professor, Institute of Cardiology, University of Latvia, Raiņa bulvāris 19, Riga, LV1002, Latvia. a.a.erglis@stradini.lv

Telephone: +371-670-69333 Fax: +371-670-69548

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Abstract

For many years, coronary angiography has been considered "the gold standard" for evaluating patients with coronary artery disease. However, angiography only provides a planar two-dimensional silhouette of the lumen and is unsuitable for the precise assessment of atherosclerosis. With the introduction of intravascular imaging, direct visualization of the arterial wall is now feasible. Intravascular imaging modalities extend diagnostic information, thereby enabling more precise evaluation of plaque burden and vessel remodeling. Of all technologies, intravascular ultrasound (IVUS) is the most mature and widely used intravascular imaging technique. Optical coherence tomography (OCT) is an evolving technology that has the highest spatial resolution of existing imaging methods, and it is becoming increasingly widespread. These methods are useful tools for planning interventional strategies and optimizing stent deployment, particularly when stenting complex lesions. We strongly support the mandatory use of IVUS for left main percutaneous coronary intervention (PCI). In addition, it can be used to evaluate vascular

responses, including neointimal growth and strut apposition, during follow-ups. Adequately powered randomized trials are needed to support IVUS or OCT use in routine clinical practice and to answer whether OCT is superior to IVUS in reducing adverse events when used to guide PCI. The current perception and adoption of innovative interventional devices, such as bioabsorbable scaffolds, will increase the need for intravascular imaging in the future.

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Key words: Imaging; Ultrasonics; Optical coherence tomography; Stent; Restenosis; Thrombosis

Core tip: Intravascular ultrasound (IVUS) and optical coherence tomography (OCT) are imaging methods that allow the direct visualization of the arterial wall and atherosclerosis. These methods are useful tools for planning interventional strategies and optimizing stent deployment and for evaluating vascular responses during follow-ups. In this review, we focus on the potential clinical utility of IVUS and OCT in patients with coronary artery disease.

Jegere S, Narbute I, Erglis A. Use of intravascular imaging in managing coronary artery disease. *World J Cardiol* 2014; 6(6): 393-404 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i6/393.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i6.393>

INTRODUCTION

Intravascular ultrasound (IVUS) is the first widely applied catheter-based imaging technology that provides valuable diagnostic information to angiography (*i.e.*, vessel and lumen dimensions, plaque burden and morphology)^[1]. IVUS uses a miniaturized ultrasound transducer mounted

on the tip of a catheter. In principle, IVUS is based on the emission, attenuation, and backscattering of ultrasonic waves that are converted to electrical signals and then processed as an image. The envelope (amplitude) of the radiofrequency signal is used to form the grey-scale IVUS image. In recent years, information derived from the spectral analysis of IVUS backscattered data has been added to grey-scale reconstructions to obtain a more detailed characterization of plaque morphology as a color-coded map^[2]. Three main post-processing methods for tissue characterization are virtual histology IVUS (VH-IVUS, Volcano Therapeutics, Rancho Cordova, CA, United States), iMAP-IVUS (Boston Scientific Corp, Fremont, CA, United States), and integrated backscatter IVUS (IB-IVUS)^[3-5]. Intravascular palpography, which measures mechanical strain of the arterial wall and has the potential to differentiate between fibrous and fatty plaque components and detect high-stress regions^[6], is a technique that is also based on IVUS. Recently, new intravascular imaging techniques with other energy sources (*e.g.*, light) have been introduced. Optical coherence tomography (OCT) is an optical technology that is based on the emission and reflection of near-infrared light. OCT has approximately 10-fold greater resolution than ultrasound-based approaches. However, the higher resolution (10 to 15- μ m axial and 20 to 25- μ m lateral) comes at the expense of poorer penetration through blood and tissue (1 to 3 mm). Recently, the earlier time-domain OCT has been replaced by frequency-domain OCT (FD-OCT) technology to reduce ischemia during blood-free optical imaging. This technique does not require proximal balloon occlusion and allows for the comprehensive scanning of long arterial segments within a few seconds^[7]. Intracoronary angioscopy is an endoscopic technology that allows direct visualization of the surface color and superficial morphology^[8]. Near-infrared spectroscopy (NIRS) uses a laser light source to detect lipid-rich plaques^[9]. A combined NIRS-IVUS catheter has recently been introduced; it provides simultaneous acquisition of grey-scale IVUS and identification of lipid core-containing plaques.

In this review, we focus on the potential clinical utility of IVUS and OCT in patients with coronary artery disease for planning interventions and percutaneous coronary intervention (PCI) guidance.

ASSESSMENT OF ANGIOGRAPHIC INTERMEDIATE LESIONS

Intravascular imaging methods enable more precise assessments of lesion severity in cases of angiographic intermediate coronary lesions. Fractional flow reserve (FFR) is the gold standard for invasive assessments of the functional significance of intermediate lesions^[10]; however, there have been attempts to correspond IVUS or OCT measurements to the functional significance of a stenosis.

Relationship between IVUS measurements and FFR

Several studies have shown good correlation between

IVUS measurements and FFR values. In a study of 53 angiographic intermediate coronary lesions, a minimum lumen area (MLA) of ≤ 4.0 mm² (by IVUS) was reported to be the best cut-off value in identifying FFR < 0.75 , with 92% sensitivity and 56% specificity^[11]. Moreover, low event rates (a mean follow-up time of 13 mo) were reported in 300 patients for whom PCI was deferred on the basis of an IVUS MLA ≥ 4.0 mm² or a minimum lumen diameter ≥ 2.0 mm, and the event rate decreased as the MLA increased^[12]. An MLA cutoff of 4.0 mm² has been the IVUS parameter that is more frequently applied in the clinical setting. However, recent studies have found different MLA cutoff values and have used a combination of other IVUS parameters to predict FFR. Recently, in a population of 201 patients with 236 coronary lesions, the best cutoff value to predict a FFR < 0.80 was an MLA < 2.4 mm², with a diagnostic accuracy of 68%, a high sensitivity of 90% and a poor specificity of 60%. Plaque burden and lesion length measured by IVUS were also the independent determinants for FFR^[13]. An IVUS-derived MLA < 2.0 mm² has been reported as the best cutoff value to predict FFR < 0.75 in vessels with reference diameters measuring < 3 mm^[14].

Few studies have validated IVUS measurements as anatomic predictors for the functional significance of left main lesions. In an analysis of 55 patients, Jasti *et al*^[15] reported that an MLA of 5.9 mm² and a minimum lumen diameter of 2.8 mm strongly predicted FFR < 0.75 . In the LITRO study, which enrolled 354 patients with intermediate left main lesions, an MLA > 6 mm² was a safe value for deferring revascularization. In the 2-year period, there was no significant difference between the deferred and revascularized groups in terms of cardiac death-free survival (97.7% *vs* 94.5%, respectively, $P = 0.5$) and event-free survival (87.3% *vs* 80.6%, respectively, $P = 0.3$)^[16]. Recently, Kang *et al*^[17] addressed this issue in 55 patients with isolated intermediate left main lesions. The IVUS MLA value that best predicted FFR < 0.80 was 4.8 mm², with 89% sensitivity and 83% specificity. In contrast with studies of non-left main stenosis, the specificity was acceptable high.

Based on this evidence, most intermediate non-left main lesions with an MLA ≥ 4 mm² are non-significant, and PCI may be deferred. However, physiological evaluation is still recommended for lesions with MLA < 4.0 mm² because of poor specificity of IVUS parameters. Other IVUS parameters should be considered in combination with the MLA to justify revascularization, including reference vessel size, lesion length, plaque burden and area stenosis. Revascularization may be deferred in patients with left main MLA ≥ 6.0 mm². FFR or non-invasive stress tests should be performed for an MLA < 6.0 mm². IVUS, therefore, should be used with caution as a tool to investigate the functional significance of intermediate lesions; the accuracy of IVUS measurements in predicting abnormal FFR remains debatable.

Recently the Society of Cardiovascular Angiography and Interventions released an expert consensus statement

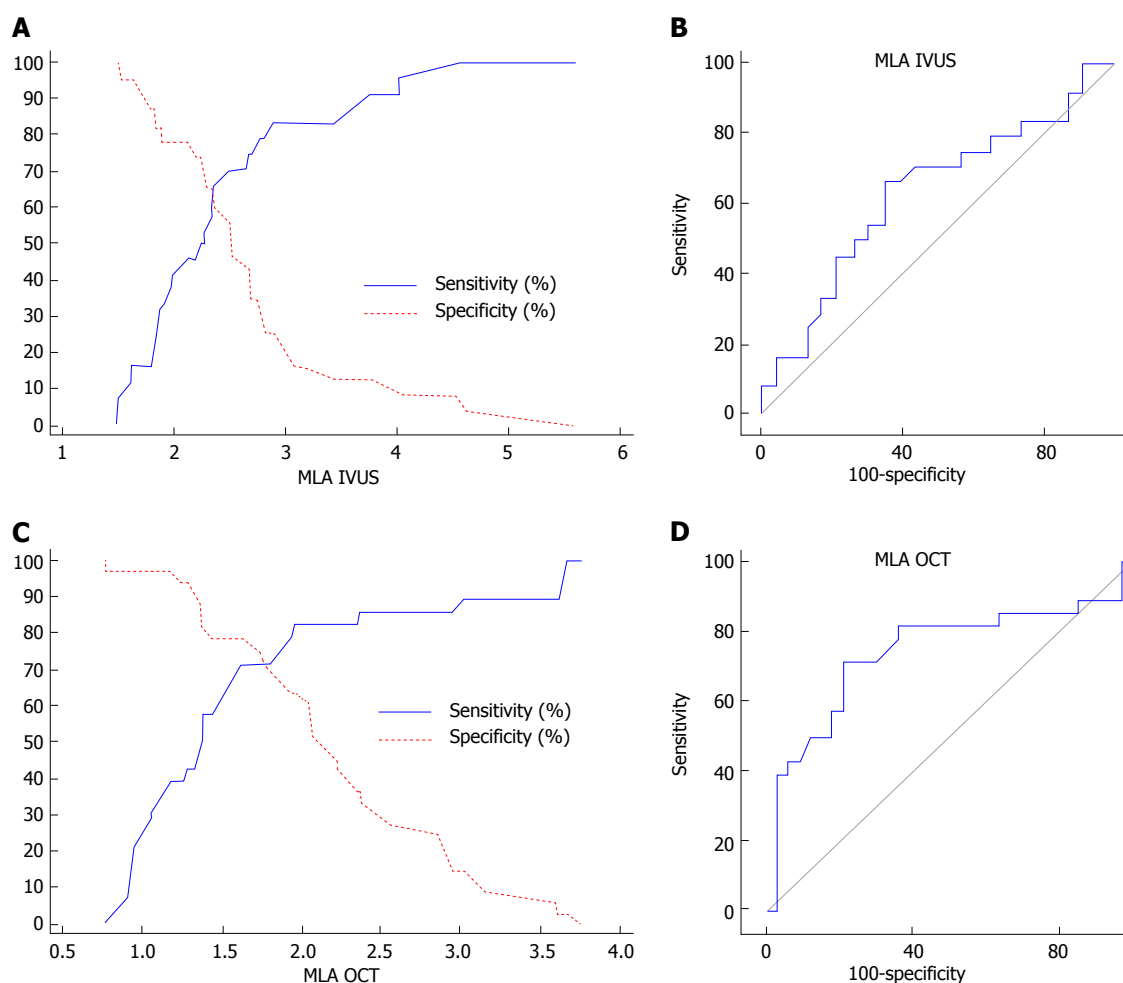


Figure 1 Intravascular ultrasound and optical coherence tomography derived minimum lumen area and fractional flow reserve. A: Sensitivity and specificity curve for IVUS-derived MLA to predict FFR ≤ 0.80 ; B: Receiver-operating characteristic curve for IVUS-derived MLA to predict FFR ≤ 0.80 ; C: Sensitivity and specificity curve for OCT-derived MLA to predict FFR ≤ 0.80 ; D: Receiver-operating characteristic curve for OCT-derived MLA to predict FFR ≤ 0.80 ^[19]. MLA: Minimum lumen area; IVUS: Intravascular ultrasound; OCT: Optical coherence tomography; FFR: Fractional flow reserve.

on the use of FFR, IVUS, and OCT. Experts recommend using IVUS to appraise the significance of left main lesions and employing a cutoff MLA value of 6.0 mm² to assess whether revascularization is warranted. However, the use of IVUS should be discouraged when evaluating non-left main lesions^[18].

Relationship between OCT measurements and FFR

Few studies have examined the potential of OCT to demonstrate the functional significance of coronary artery disease and the new expert statement does not recommend using OCT to determine stenosis functional significance^[18]. Recently, one study of 56 patients with 61 non-left main intermediate stenoses analyzed the value of OCT in identifying hemodynamically significant stenosis using FFR as a standard of reference. OCT showed moderate diagnostic efficiency in identifying coronary stenoses with FFR ≤ 0.80 (area under the curve 0.74; 95%CI: 0.61-0.84). The best OCT-derived measurements to predict FFR ≤ 0.80 were 1.95 mm² for the MLA (82% sensitivity, 63% specificity, and 72% accuracy) and 1.34 mm for the minimum lumen diameter (82% sensitivity, 67% specificity, and 73% accuracy). In addition 77% of

the stenoses were studied with IVUS. The IVUS cut-off value for MLA was 2.36 mm² (67% sensitivity, 65% specificity, and 66% accuracy). In patients with simultaneous IVUS and OCT, there were no significant differences in the diagnostic efficiency of OCT and IVUS, but in a subgroup of small vessels (reference diameter < 3 mm), OCT showed a significantly better diagnostic efficiency (Figure 1)^[19]. The moderate diagnostic efficiency demonstrated by OCT and IVUS in this study may be related to the reference diameter of 2.60 ± 0.6 mm, and 49.2% of the target vessels had reference diameters measuring < 2.5 mm. Thus, although an OCT-derived MLA may be a useful criterion for excluding hemodynamically significant stenoses, direct FFR measurements or stress tests may be necessary to identify the ischemia-inducible lesion.

INTRAVASCULAR IMAGING FOR PCI GUIDANCE

Pre-intervention imaging provides valuable information regarding the severity of stenosis, lesion length, vessel size, and plaque characteristics. It has been used to plan

Table 1 Intravascular ultrasound criteria for optimal stent deployment

MUSIC criteria
Complete apposition of the stent over its entire length against the vessel wall
MLA:
In-stent MLA $\geq 90\%$ of the average reference lumen area or $\geq 100\%$ of the reference segment with the lowest lumen area
In-stent MLA of proximal stent entrance $\geq 90\%$ of proximal reference lumen area
If the in-stent MLA is $> 9.0 \text{ mm}^2$:
In-stent MLA $\geq 80\%$ of the average reference lumen area or $\geq 90\%$ of the reference segment with the lowest lumen area
In-stent MLA of proximal stent entrance $\geq 90\%$ of the proximal reference lumen area
Symmetric stent expansion defined by the minimum lumen diameter divided by the maximum lumen diameter ≥ 0.7
AVIO study criteria
Final minimum stent cross sectional area of at least 70% of the hypothetical cross sectional area of the fully inflated balloon used for post-dilatation
The optimal balloon size that should be used for post-dilatation is the average of the media to media diameters of the distal and proximal stent segments, as well as at the sites of maximal narrowing within the stent. The value is rounded to the lowest 0.00 or 0.50 mm. For values $\geq 3.5 \text{ mm}$, the operator could downsize the balloon diameter based on clinical judgment

MUSIC: Multicenter Ultrasound Stenting in Coronaries Study; MLA: Minimum lumen area; AVIO: Angiography *vs* IVUS Optimization; IVUS: Intravascular ultrasound.

and guide PCI and also provides information on the extent of calcium, the need for vessel preparation and the selection of device size and type. The presence of circumferential calcium can lead to plaque pretreatment with rotablation or cutting/scoring balloon prior to stent implantation. Post-intervention imaging has the potential to detect PCI complication, including the presence of edge dissections and plaque protrusion. It verifies stent expansion and apposition, as well as the need for post-dilatation or additional stent implantation. Randomized clinical studies of IVUS guidance for stent implantation have used various criteria to define an optimal result (Table 1)^[20,21].

Impact of IVUS on restenosis and adverse events

Several post-intervention IVUS findings have been associated with restenosis and stent thrombosis. Smaller post-procedure lumen dimensions, residual reference segment stenosis, stent underexpansion, thrombus and dissections have been reported to be IVUS predictors of restenosis or stent thrombosis^[22-25].

Stent underexpansion has been the most important mechanism of stent failure (Figure 2). In a large study of 550 patients treated with sirolimus-eluting stent implantation, the target IVUS criterion for stent expansion was a post-procedural final in-stent MLA measuring $\geq 5.0 \text{ mm}^2$ more than the distal reference segment lumen area. The only independent predictors of angiographic restenosis were final in-stent MLA by IVUS (OR = 0.586, 95%CI: 0.387-0.888, $P = 0.012$) and IVUS-measured stent length (OR = 1.029, 95%CI: 1.002-1.056, $P = 0.035$). The final in-stent MLA that best predicted restenosis was 5.5 mm^2 ^[26]. In IVUS substudies of the TAXUS IV, V, and VI and TAXUS ATLAS Workhorse, Long Lesion, and Direct Stent trials, which comprised 1580 patients, the optimal thresholds of post-intervention IVUS in-stent MLA that best predicted angiographic in-stent restenosis at 9 mo were 5.7 mm^2 for paclitaxel-eluting stents and 6.4 mm^2 for bare metal stents (BMS)^[27]. Consistent with these observations, the optimal post-intervention in-stent MLA to predict angiographic restenosis of the

second generation drug-eluting stents was 5.3 mm^2 for zotarolimus-eluting stents and 5.4 mm^2 for everolimus-eluting stents^[28]. However, a single cutoff value to define optimal stent implantation or to predict restenosis should be used cautiously because these studies enrolled patients with different risks for restenosis or lesion complexity.

Recently, Kang *et al.*^[29] reported the best IVUS-MLA criteria that predicted angiographic in-stent restenosis on a segmental basis after left main intervention. Underexpansion was defined as post-stenting IVUS-MLA $< 5.0 \text{ mm}^2$ at the ostial left circumflex, $< 6.3 \text{ mm}^2$ at the ostial left anterior descending, $< 7.2 \text{ mm}^2$ at the polygon of confluence, and $< 8.2 \text{ mm}^2$ at the proximal left main above the polygon of confluence. Post-stenting underexpansion was an independent predictor of 2-year major adverse cardiac events, particularly repeat revascularization, while stent malapposition did not predict restenosis or major adverse cardiac events.

Few studies have reported stent malapposition as a predictor of early^[30] or very late stent thrombosis^[31]. However, several IVUS studies have failed to identify incomplete stent apposition as a predictor of clinical adverse events^[32,33]. The IVUS substudy of the HORIZONS-AMI trial reported smaller final lumen dimensions because of tissue protrusion through stent struts and/or stent underexpansion and inflow/outflow disease (residual stenosis or stent edge dissections) but not acute malapposition as a predisposing factor of early stent thrombosis in acute myocardial infarction^[34].

IVUS-guided PCI

In the pre-drug-eluting stent era, several studies assessed whether IVUS-guided stent implantation improves clinical outcomes compared with standard, angiography-guided PCI. However, these studies enrolled relatively small numbers of patients and were underpowered to definitively assess the role of IVUS guidance on clinical endpoints. In a meta-analysis of 7 randomized trials ($n = 2193$) IVUS-guided BMS implantation was associated with a significantly lower rate of angiographic restenosis compared with angiographic-guided strategy (22% *vs*

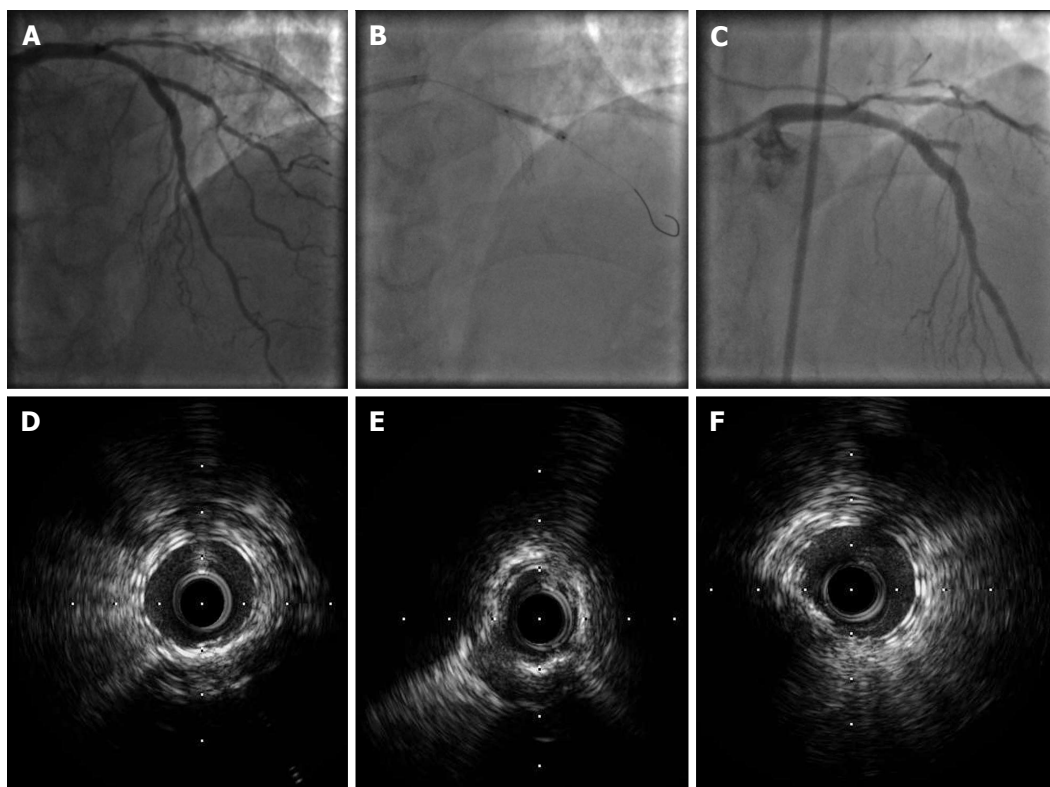


Figure 2 Intravascular ultrasound findings in patient with stent failure. A: Left anterior descending-Diagonal bifurcation treated with everolimus-eluting stent implantation in the left anterior descending and bioabsorbable everolimus-eluting scaffold implantation (T-stenting) in the diagonal branch; B: Post-dilatation with a noncompliant balloon in the diagonal branch; C: Four days later, the patient presented with acute myocardial infarction and stent thrombosis in the diagonal branch; D-E: Post-intervention IVUS showed stent underexpansion in the mid part of the diagonal branch (E) with good stent expansion at the proximal part (D) and at the distal part (F) of the diagonal branch. IVUS: Intravascular ultrasound.

29%, respectively, $P = 0.02$), with no significant effect for myocardial infarction (3.6% *vs* 4.4%, respectively $P = 0.51$) or mortality (2.4% *vs* 1.6%, respectively, $P = 0.18$)^[35]. In a larger meta-analysis of 2972 patients, IVUS-guided strategy demonstrated a reduced risk of binary restenosis, repeat revascularization and major adverse cardiac events, without significant benefits in death or myocardial infarction^[36].

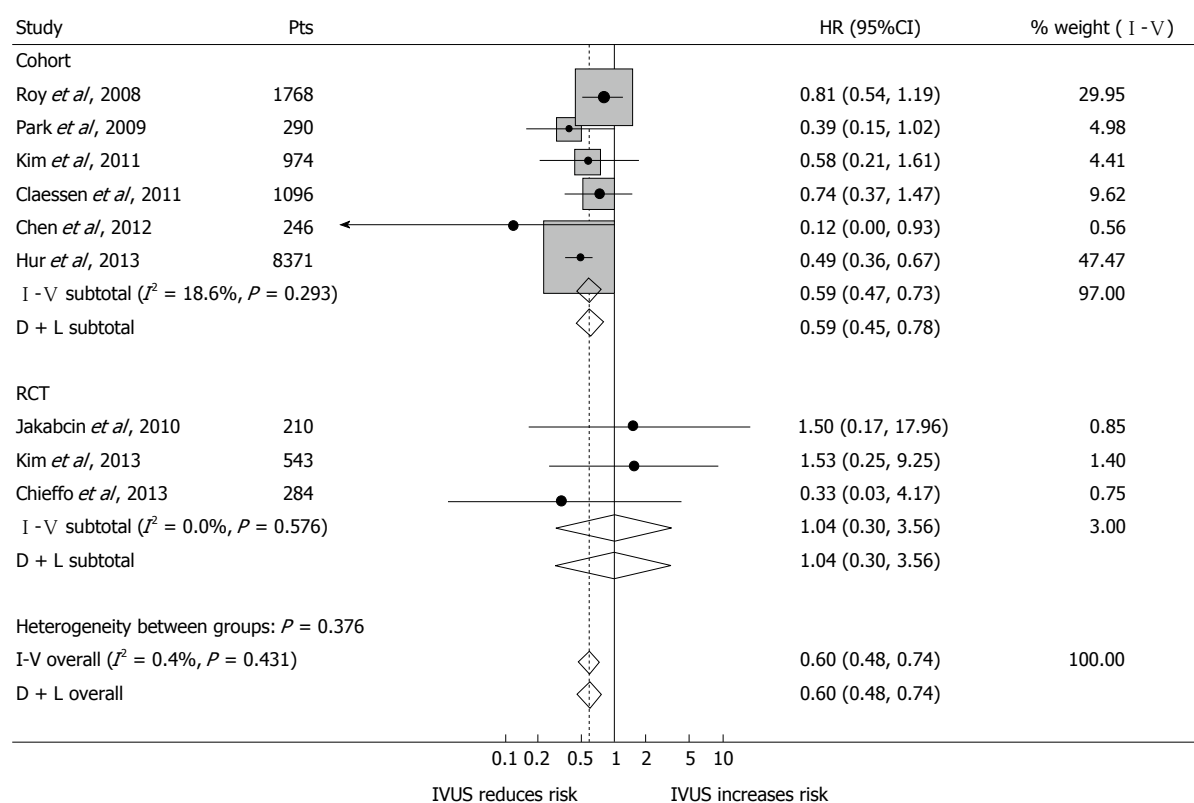
In the drug-eluting stent (DES) era, limited data from randomized trials on IVUS-guided DES are available. Recently, the Angiography *vs* IVUS Optimization (AVIO) study evaluated the safety and efficacy of IVUS *vs* angiography-guided DES post-dilatation in 284 patients with complex lesions (bifurcation, long lesions, chronic total occlusions or small vessels). IVUS guidance showed a larger final in-lesion minimum lumen diameter ($2.70 \text{ mm} \pm 0.46 \text{ mm}$ *vs* $2.51 \pm 0.46 \text{ mm}$, $P = 0.0002$), with no impact on major adverse cardiac events or target lesions revascularization at 24 mo. However, an angiographic follow-up was performed in only one-third of the patients, and in this group the restenosis rates were 17.5% in the IVUS group and 28.6% in the angiography group. Moreover, the top enrollment centers had substantial experience with IVUS, and operators may develop an “IVUS eye” that leads to the ability to perform aggressive post-dilatation even with angiography guidance alone^[21]. A meta-analysis of 18707 patients from 3 randomized

IVUS *vs* angiography-guided studies and 9 high quality cohort studies found that IVUS guidance reduced the risk of major adverse cardiac events (RR = 0.80, 95%CI: 0.71-0.89, $P = 0.001$). This technique was associated with a reduced risk of mortality (RR = 0.60, 95%CI: 0.48-0.74, $P = 0.001$), myocardial infarction (RR = 0.59, 95%CI: 0.44-0.80, $P = 0.001$) and thrombosis (RR = 0.50, 95%CI: 0.32-0.80, $P = 0.007$) but not of revascularization (RR = 0.95, 95%CI: 0.82-1.09, $P = 0.75$) (Figure 3)^[37]. This meta-analysis is supported by a recently published large-scale prospective, multicenter, non-randomized ADAPT-DES study of 8583 “all-comers” patients. In propensity adjusted multivariable analysis, IVUS guidance compared to angiography reduced the risk of stent thrombosis (0.6% *vs* 1.0%, respectively, $P = 0.003$), myocardial infarction (2.5% *vs* 3.7%, respectively, $P = 0.004$) and major adverse cardiac events (3.1% *vs* 4.7%, respectively, $P = 0.002$) within 1 year following DES implantation^[38]. IVUS guidance was particularly beneficial among patients with acute coronary syndromes and complex lesions, including left main, bifurcations and multivessel disease. In contrast, Ahmed *et al*^[39] reported that the use of IVUS guidance for stent deployment failed to improve 12-mo mortality rates in patients presenting with acute myocardial infarction.

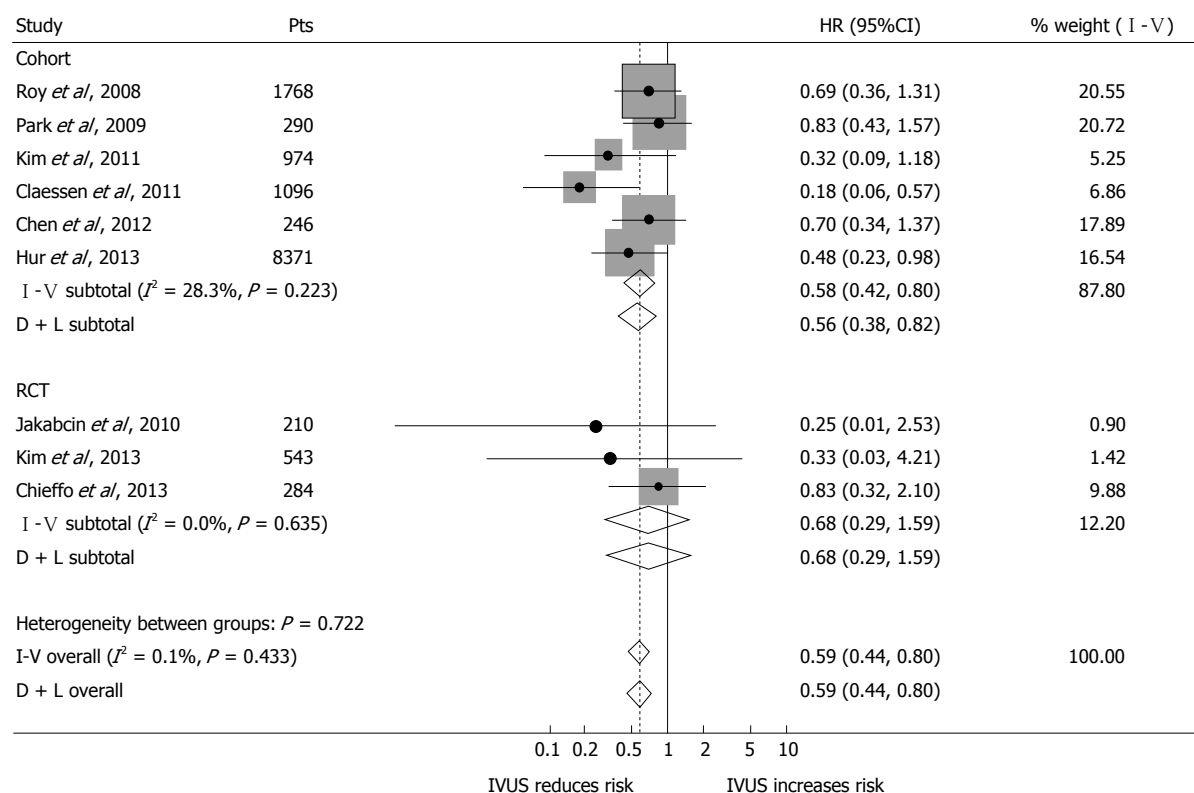
IVUS-guided PCI of left main lesions

In the MAIN-COMPARE multicenter registry, 975 pa-

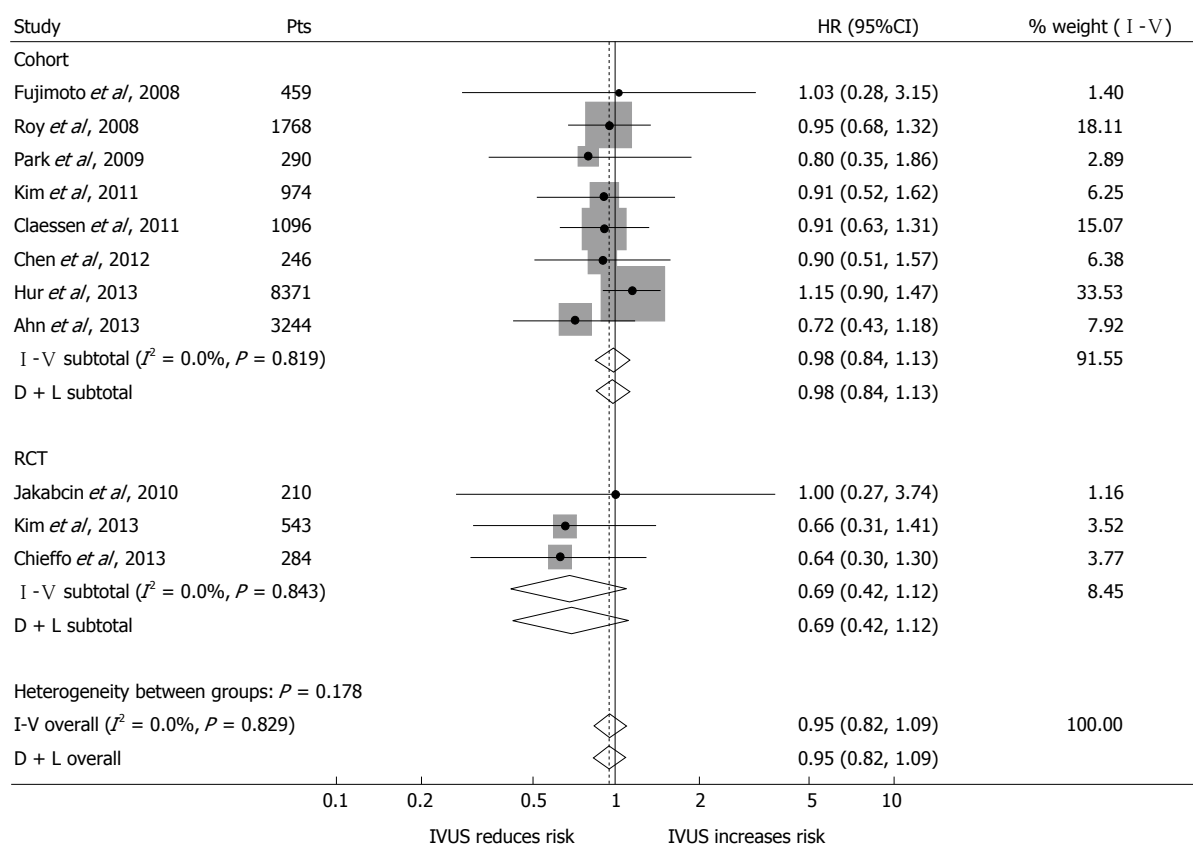
IVUS and dead (primary population)



IVUS and MI (primary population)



IVUS and TVR_TLR (primary population)



IVUS and thromb (primary population)

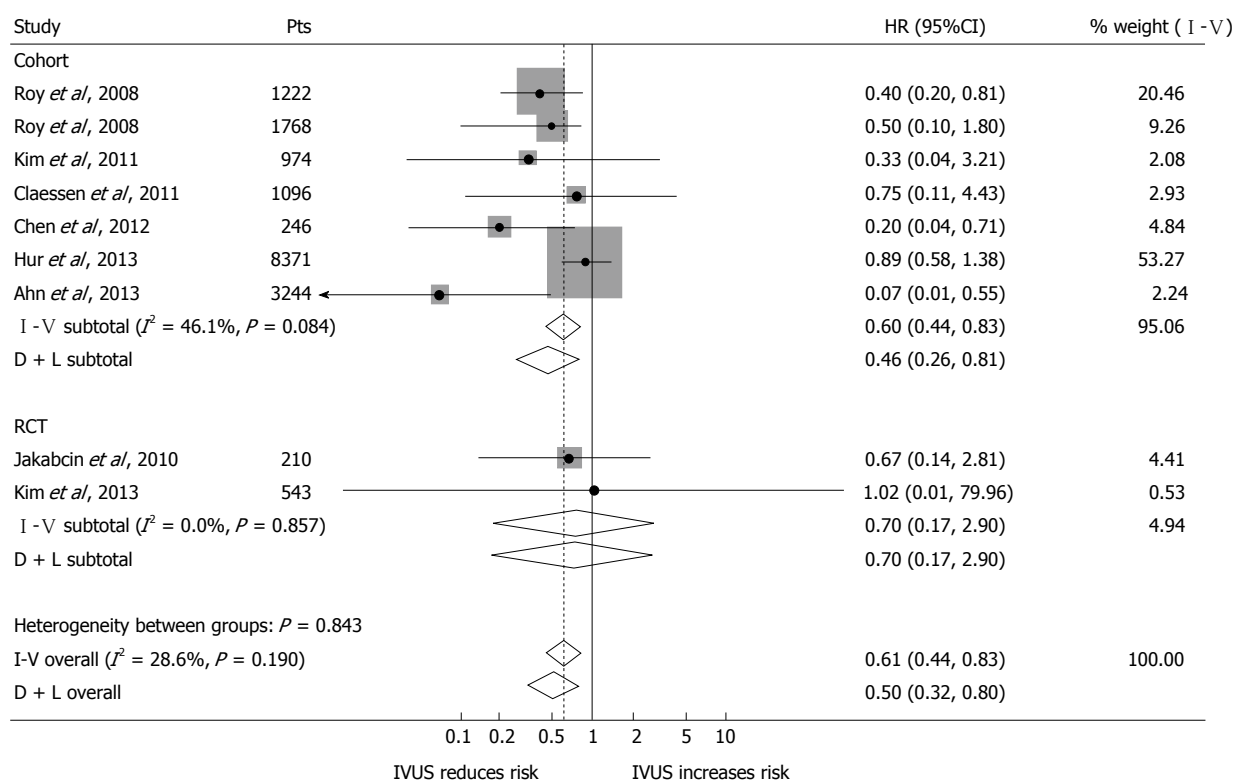


Figure 3 Impact of intravascular ultrasound vs angiography guidance of percutaneous coronary intervention on clinical outcomes. A Forrest plot of the secondary endpoints [*i.e.*, death, myocardial infarction (MI), target vessel and lesion revascularization (TVR_TLR), thrombosis]. Diamonds represent the meta-analytic estimates and 95%CI. Adapted from [37]. IVUS: Intravascular ultrasound.

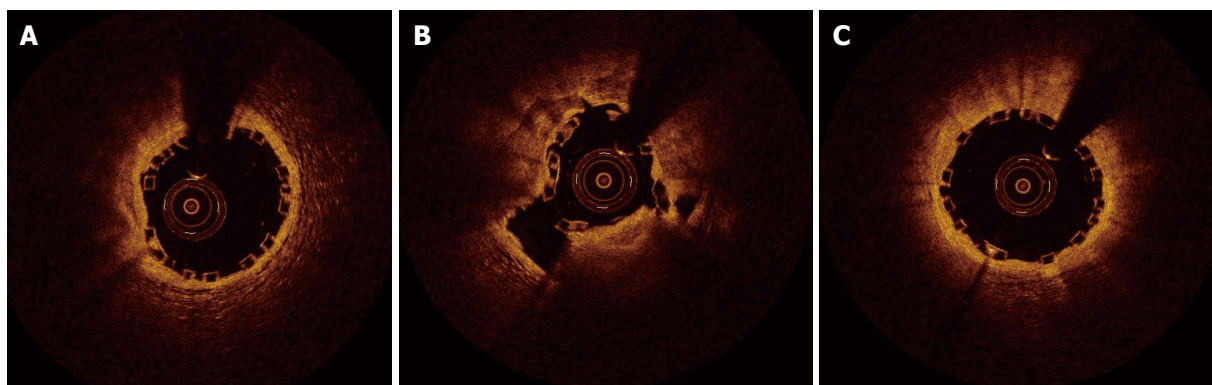


Figure 4 Optical coherence tomography findings in patient with stent underexpansion. A-C: Post-intervention OCT of the diagonal branch after bioabsorbable scaffold implantation in a patient who presented 4 d later with stent thrombosis and acute myocardial infarction. OCT showed stent underexpansion of the mid part of the diagonal branch (B) with good stent expansion at the proximal (A) and distal (C) part of the diagonal branch. OCT: Optical coherence tomography.

tients with unprotected left main coronary artery stenosis underwent PCI under the guidance of IVUS or angiography alone. In the propensity-score matched comparison, IVUS guidance showed a trend towards lower 3-year mortality rates (6.0% in the IVUS group *vs* 13.6% in the angiography group, log-rank $P = 0.063$; HR = 0.54; 95%CI: 0.28-1.03; Cox-model $P = 0.061$). In particular, patients receiving DES had significantly lower mortality rates with IVUS guidance (4.7% *vs* 16.0%, log-rank $P = 0.048$; HR = 0.39; 95%CI: 0.15-1.02; Cox model $P = 0.055$), but after BMS implantation, the IVUS guidance did not reduce the risk of death^[40]. Our Latvian randomized trial comparing paclitaxel-eluting stents to BMS in treating unprotected left main coronary artery stenosis demonstrated that PCI with IVUS guidance and cutting balloon pre-treatment is safe and effective for up to 3 years after intervention^[41,42]. Therefore, we strongly support the mandatory use of IVUS for left main PCI.

Although large prospective studies appear to support IVUS-guided DES implantation, randomized trials have been underpowered to definitively assess the clinical utility of IVUS guidance because of their small sample sizes and low event rates, including restenosis or highly morbid complications.

OCT-guided PCI

OCT has evolved from time-domain to frequency-domain imaging, which does not require proximal balloon occlusion and allows imaging of long coronary segment in a few seconds. OCT provides greater resolution than IVUS and excellent contrast between lumen and vessel wall imaging. Therefore, OCT can assess coronary plaque morphologies and identify suboptimal stent failure (*e.g.*, incomplete stent apposition, intrastent tissue protrusion, stent edge dissection, and intrastent thrombus) that is missed by IVUS. Similar to IVUS, OCT can be used to identify stent underexpansion (Figure 4). In 73 consecutive patients (80 vessels) evaluated by OCT, the incidence of edge dissection was 25%, but this incidence were not associated with clinical events during hospitalization^[43]. The clinical significance of edge dissections and other parameters identified by OCT must be addressed by pro-

spective trials.

FD-OCT provides more accurate quantitative analysis of lumen. In the OPUS-CLASS study, the *in vivo* minimum lumen diameter and area measured by FD-OCT was significantly greater than those measured by quantitative coronary angiography (QCA) but smaller than those measured by IVUS. In a phantom model, the mean lumen area by FD-OCT was equal to the actual lumen area of the phantom model, while IVUS overestimated the area measurements^[44]. The difference in lumen measurements between the 2 techniques is likely caused by the superior ability of FD-OCT to visualize the lumen-intima interface. Therefore, caution should be exercised before using the recommended IVUS parameters to assess lesion significance and to guide PCI by FD-OCT. The disadvantage of OCT is its limited far-field penetration. Thus, it may be more difficult to measure the true vessel size (external elastic membrane) and to identify a landing zone with the smallest plaque burden to minimize geographical miss.

In the CLI-OPCI study, Prati *et al*^[45] compared OCT guidance on top of angiography for routine PCI to angiographic guidance alone in 670 patients. OCT guidance was associated with a significantly lower risk of cardiac death (3.3% *vs* 6.9%, respectively, $P = 0.035$) and the composite of cardiac death, myocardial infarction, or repeat revascularization at 1 year. Thus, OCT is a safe and feasible tool for PCI guidance. However, further investigations are needed to confirm whether the use of FD-OCT will improve clinical outcomes.

OCT vs IVUS for PCI guidance

There are ongoing discussions as to whether FD-OCT has the potential to replace IVUS for PCI guidance. In a small prospective, single center study of 70 patients, FD-OCT guidance was compared with IVUS guidance for coronary stent implantation. Although both devices showed similar accessibility and there was no significant difference for stent apposition, FD-OCT guidance demonstrated a smaller final minimum stent area, as well as smaller stent expansion and more frequent significant residual reference segment stenosis. Researchers con-

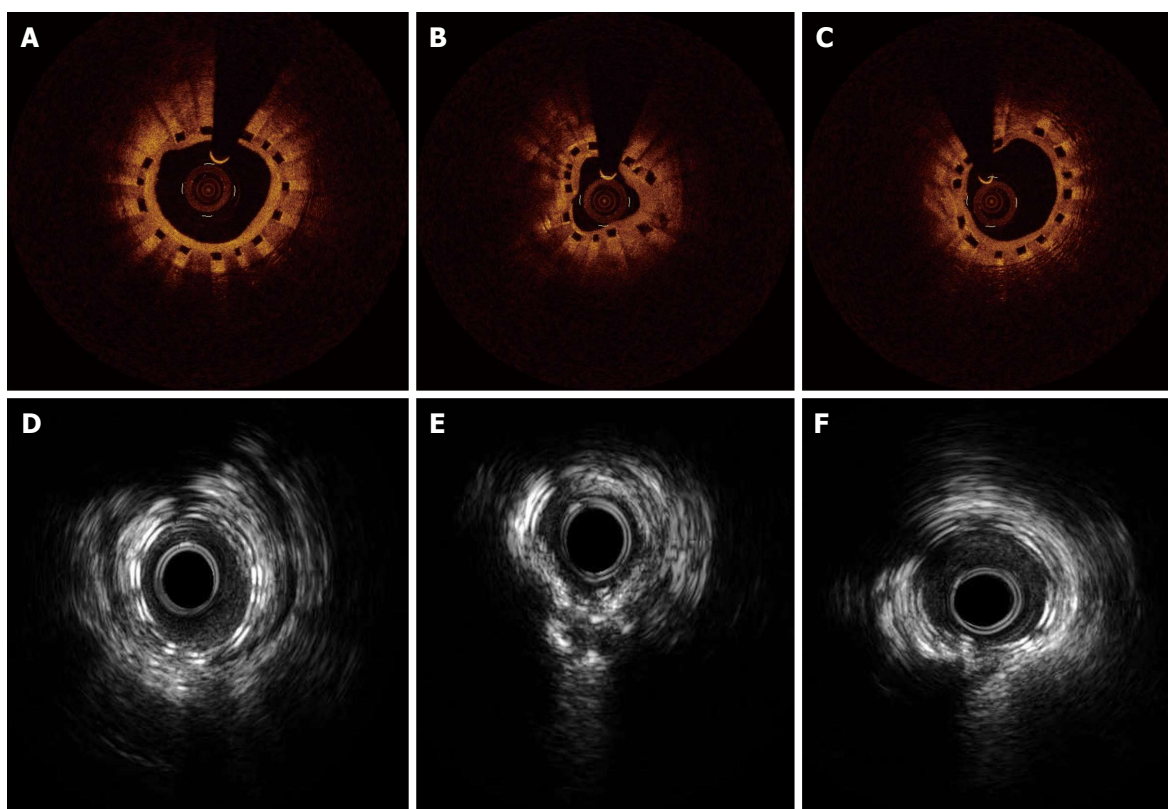


Figure 5 Intravascular ultrasound and optical coherence tomography findings 1 yr after bioabsorbable stent implantation. A-C: The OCT findings 12 mo after bioabsorbable scaffold implantation showed complete strut coverage; D-E: IVUS also shows uncovered struts in the same patient. IVUS: Intravascular ultrasound; OCT: Optical coherence tomography.

cluded that OCT has several limitations for optimal stent deployment because of the poor visibility of the vessel border. Good vessel border visibility at the MLA site was more frequently observed in the IVUS group both prior to intervention (94.3% *vs* 8.6%, $P < 0.001$) and post-intervention (94.3% *vs* 11.4%, $P < 0.001$). This difference in visibility resulted in a lower frequency of post-dilatation and lower stenting and post-dilatation pressure in the OCT group^[46]. Further studies are warranted to determine whether IVUS or OCT is better suited to improve clinical outcomes after stent implantation.

EVALUATION OF NEOINTIMAL COVERAGE AFTER PCI

Intravascular imaging methods have been used to assess the vascular response to stent implantation during follow-up. Endothelial coverage is a powerful histological predictor of stent thrombosis. Post-mortem studies have shown that uncovered struts are strongly associated with late stent thrombosis^[47]. With the introduction of OCT, it is possible to perform strut level analysis and to evaluate neointimal growth and stent apposition on each stent strut. Because OCT has higher resolution compared to IVUS, it is more sensitive for detailed strut-level analysis of tissue coverage and apposition (Figure 5). Stent struts are classified on OCT into four main categories: embedded-covered, protruding-covered, uncovered-

apposed, uncovered malapposed struts. In a subanalysis of the ODESSA trial, 8% of the stented segments with no detectable neointimal coverage by IVUS were found to have tissue coverage of the stent struts by OCT^[48]. In a study of 34 patients (6840 struts), the prevalence of struts covered by neointima that were undetectable by IVUS was 64% at the 6-mo follow-up after sirolimus-eluting stent implantation. A total of 16% of the struts showed full coverage by neointima, whereas the average rate of neointima-covered struts in an individual stents was 89%^[49]. In a formal substudy of HORIZONS-AMI trial, OCT was performed at 13 mo in 118 patients after paclitaxel-eluting stent or BMS implantation. An analysis of 44139 struts revealed reduced neointimal hyperplasia and a greater percentage of uncovered struts, as well as higher percentage of malapposed struts in paclitaxel-eluting stents compared with BMS. While these observations are important in term of stent design, further studies are needed to determine the clinical significance of these findings^[50].

OCT also plays a critical role in assessing bioabsorbable scaffolds. OCT is capable of an accurate assessment of polymeric struts, which are seen as “boxes”, scaffold degradation and neointimal formation at follow-up^[51].

CONCLUSION

Compared to angiography, intravascular imaging provides additional anatomic information regarding vessel wall

changes in atherosclerosis, but these methods should be used cautiously for the physiologic assessment of coronary artery disease. Therefore, the use of intravascular imaging and FFR should be complementary to guide decision making in certain coronary lesions. Because of their excellent imaging quality and spatial resolution, IVUS and OCT are the best tools for evaluating optimal stent deployment. Successful PCI of complex lesions often requires IVUS guidance, novel devices and advanced operator skills. The current perception and adoption of innovative interventional devices, such as bioabsorbable stents, will increase the need for intravascular imaging. Today, the routine use of intravascular imaging in daily practice remains controversial. Adequately powered randomized trials are needed to support IVUS or OCT use in routine clinical practice and to determine whether OCT is superior to IVUS in reducing adverse events when used to guide PCI. Selective angiography will remain vital for managing coronary artery disease. Intravascular modalities will complement rather than replace this “gold standard” and will be routinely used in selected patients. The future of intravascular imaging is the integration of functional and anatomical assessment and the usage of multiple imaging modalities in a complementary manner to diagnose and manage coronary artery disease.

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WJC 6th Anniversary Special Issues (2): Coronary artery disease

Role of cardiovascular magnetic resonance in assessment of acute coronary syndrome

Shah M Azarisman, Karen S Teo, Matthew I Worthley, Stephen G Worthley

Shah M Azarisman, Department of Internal Medicine, Kulliyyah of Medicine, International Islamic University Malaysia, Bandar InderaMahkota, 25200 Kuantan, Pahang, Malaysia

Shah M Azarisman, Karen S Teo, Matthew I Worthley, Stephen G Worthley, Cardiovascular Research Centre, Royal Adelaide Hospital, North Terrace, Adelaide 5000, Australia

Author contributions: Azarisman SM and Worthley SG designed and wrote the paper; Teo KS and Worthley MI co-wrote, proof read and edited the paper.

Correspondence to: Dr. Shah M Azarisman, Department of Internal Medicine, Kulliyyah of Medicine, International Islamic University Malaysia, Jalan Sultan Abdullah, Bandar InderaMahkota, 25200 Kuantan, Pahang, Malaysia. risman1973@hotmail.com

Telephone: +60-13-9230110 Fax: +60-13-9230110

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Abstract

Cardiovascular disease (CVD) is the leading cause of death in the western world and is becoming more important in the developing world. Recently, advances in monitoring, revascularisation and pharmacotherapy have resulted in a reduction in mortality. However, although mortality rates have declined, the burden of disease remains large resulting in high direct and indirect healthcare costs related to CVDs. In Australia, acute coronary syndrome (ACS) accounts for more than 300000 years of life lost due to premature death and a total cost exceeding eight billion dollars annually. It is also the main contributor towards the discrepancy in life expectancy between indigenous and non-indigenous Australians. The high prevalence of CVD along with its associated cost urgently requires a reliable but non-invasive and cost-effective imaging modality. The imaging modality of choice should be able to accelerate the diagnosis of ACS, aid in the risk stratification of de novo coronary artery disease and avail incremental

information of prognostic value such as viability which cardiovascular magnetic resonance (CMR) allows. Despite its manifold benefits, there are limitations to its wider use in routine clinical assessment and more studies are required into assessing its cost-effectiveness. It is hoped that with greater development in the technology and imaging protocols, CMR could be made less cumbersome, its imaging protocols less lengthy, the technology more inexpensive and easily applied in routine clinical practice.

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Key words: Cardiovascular disease; Acute coronary syndrome; Cardiac imaging

Core tip: This review focuses on cardiovascular magnetic resonance in achieving speedy diagnosis, risk stratification and prognostication in acute coronary syndrome. It discusses the modalities already available towards achieving this end and the incremental information availed by cardiac magnetic resonance. The paper also discusses new imaging techniques and their contribution towards the cardiac magnetic resonance imaging assessment of patients with acute coronary syndrome.

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INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in the western world and is becoming more im-

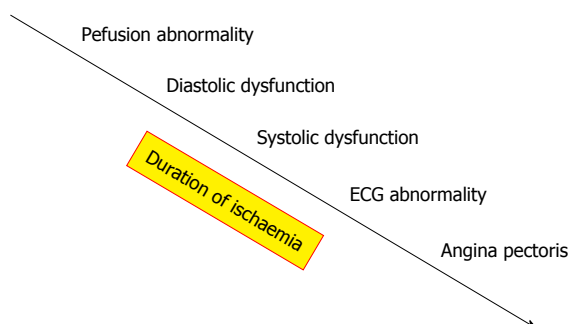


Figure 1 Cascade of events following coronary artery occlusion. (Adapted from Gani *et al*^[14]). ECG: Electrocardiography.

portant in the developing world^[1,2]. Recently, advances in monitoring, revascularisation and pharmacotherapy have resulted in a reduction in mortality. However, although mortality rates have declined, the burden of disease remains large resulting in high direct and indirect healthcare costs related to CVDs^[3-5]. In Australia, acute coronary syndrome (ACS) accounts for more than 300000 years of life lost due to premature death and a total cost exceeding eight billion dollars annually. It is also the main contributor towards the discrepancy in life expectancy between indigenous and non-indigenous Australians^[6]. In the United States and Europe, approximately 15 million patients are treated annually for chest pain and suspicion of myocardial infarction (MI) and upwards of 20% are eventually diagnosed to have ACS^[2,7].

The high prevalence of CVD along with its associated cost urgently requires a reliable but non-invasive and cost-effective imaging modality. The imaging modality of choice should be able to accelerate the diagnosis of ACS, aid in the risk stratification of de novo coronary artery disease (CAD) and avail incremental information of prognostic value such as viability.

ACUTE CORONARY SYNDROME

It is well established that ACS refers to a spectrum of clinical presentations ranging from unstable angina to non ST-elevation myocardial infarction and ST-elevation myocardial infarction. These presentations refer to clinical symptoms compatible with myocardial ischaemia resulting from acute thrombosis induced by a ruptured or eroded atherosclerotic coronary artery plaque^[1-4].

The main management strategy for ACS is prompt diagnosis leading to early coronary reperfusion. The usual assessment sequence involves a detailed case history delineating the patient's risk factor profile, appropriate physical examination, electrocardiography (ECG) and laboratory risk markers such as creatine kinase and troponin levels.

Early reperfusion limits the final infarct size, halts progression of myocardial necrosis and and optimises myocardial salvage thereby improving both short and long term outcomes^[8,9]. Pertaining to these established aims, several questions need to be answered. What is the regional and global ventricular function, what is the

extent of myocardial necrosis, is there any viable myocardium and are the epicardial coronary arteries patent?^[10-12].

Over the past two decades, noninvasive imaging has emerged as the investigative modality of choice for ACS. It allows comprehensive cardiac assessment of patients, risk stratification of patients with ACS at an early management time point and provides diverse and complementary information regarding possible differential diagnoses and prognosis^[13-15].

NONINVASIVE ASSESSMENT

Early coronary reperfusion following diagnosis of ACS results in myocardial salvage and prevents irreversible injury^[16,17]. Usual investigative tools such as ECG and Troponin assays are helpful but may be negative early. Echocardiography, although useful in establishing regional wall motion abnormalities and quantifying ventricular ejection fraction, can also be negative early as these abnormalities appear later in the temporal cascade of events following coronary artery occlusion (Figure 1). Furthermore, echocardiographic assessment lacks the tissue characterisation ability needed to rule out differentials such as myocarditis. Over the past two decades, computed tomography (CT) has emerged as a potentially useful imaging modality for ACS.

COMPUTED TOMOGRAPHY BASED IMAGING

Positron emission tomography

Positron emission tomography (PET) utilises several radionuclides namely ¹⁸F-Fluorodeoxyglucose (¹⁸FDG) for myocardial metabolism and ¹³N-Ammonia (¹³NH₃) for myocardial perfusion assessment^[18]. Myocardial segments with normal glucose metabolism and preserved myocardial flow indicate viable and adequately perfused myocardium. ¹⁸FDG allows differentiation between hibernating but viable, with infarcted and non-viable myocardium in regions with wall motion abnormalities when interpreted together with ¹³NH₃^[19,20]. Although clinically useful in identifying metabolism/perfusion mismatch in stable CAD, its utility in the setting of ACS is limited due to restricted availability, high costs, and limited data supporting its application^[21].

Coronary angiography

Computed tomography coronary angiogram (CTCA) is becoming a useful tool for evaluation of patients with ACS. It can be utilised both in the diagnosis and risk stratification of ACS^[22,23]. Three recent trials affirmed the utility of employing CTCA for rapid triage *via* radiographic demonstration of the absence of coronary artery disease in low to intermediate risk patients^[24-26]. Whilst all three trials reported more rapid and cost efficient discharge from the Emergency Department with the use of CTCA, the CT-STAT and ROMICAT II trials reported an increase in downstream testing and radiation exposure

with no decrease in the overall costs of care^[25,26]. Although the appropriate use criteria endorses its use in low to intermediate risk patients, it is primarily an exclusion tool with limited suitability for higher cardiac risk patients or pathological stress testing^[27].

Calcium score

Coronary artery calcification can be evaluated by electron beam CT and multi-detector CT. It describes the extent of coronary atherosclerosis and is correlated with increased cardiac risk. It has a high negative predictive value, and can reliably exclude ACS in low to intermediate risk patients presenting with chest pain^[28-30]. Unfortunately, its positive predictive value is unsatisfactory and a positive result usually warrants further downstream investigation. Moreover, conclusive evidence on its use in conjunction with other CT modalities such myocardial perfusion imaging (MPI) is still deficient^[14,31].

MPI

Rest MPI becomes abnormal at the onset of impaired myocardial blood flow and therefore precedes other symptoms and signs of ACS. The non-invasive detection of a resting perfusion defect can be achieved with single-photon emission CT (SPECT), PET, cardiovascular magnetic resonance (CMR) and contrast enhanced echocardiography^[32-34].

Resting myocardial perfusion is preserved with increasing severity of coronary stenosis through autoregulatory mechanisms in the microcirculation. This is exhausted when critical coronary artery stenosis develops and a resting myocardial perfusion abnormality will appear with complete occlusion of the coronary artery^[35].

Cardiac CT based MPI has been utilised in animals since the late 1970s but its use in detection of MI only took off in mid-2000^[36-38]. Resting MPI in addition to CTCA improves its diagnostic accuracy for detecting significant coronary artery disease. Studies have shown that in patients with chest pain, MPI with CTCA helps clarify the diagnosis of ACS^[39-41]. Unfortunately rest MPI is not sensitive enough to identify the majority of ischaemic segments and vasodilator-induced hyperemia is required to detect significant disease^[42-44].

Stress MPI detects the presence of a flow-limiting coronary stenosis by detecting regional variations in perfusion reserve. During vasodilator-induced hyperaemia, blood flow will not increase in already dilated arteriolar bed of stenosed coronary arteries. However, perfusion of normal coronaries will increase significantly and the resultant increase over resting blood flow is referred as the perfusion reserve. Consequently, the perfusion reserve of normal coronary territories will be greater than that of critically stenosed coronary territories and this regional discrepancy is detected by stress MPI^[32-34].

Stress MPI is especially helpful in patients with coronary calcification and stents, with studies reporting a sensitivity and specificity of at least 95%^[45,46]. Most studies however, report a sensitivity of between 50%-90%

and specificity of 50%-98% when compared with either SPECT, CMR or invasive fractional-flow reserve (FFR) studies^[32,45-50].

The major limitation to CT based rest and stress MPI, as with other CT based modalities, especially in research with comprehensive protocols remains exposure to ionizing radiation. Lack of long-term follow-up data of patients presenting to Emergency Department with chest pain and subsequently diagnosed with ACS is also compelling. Furthermore, although more recent studies have shown greater ability of different CT-based modalities in diagnosing and risk stratifying ACS, their utility remains only with those in the low to intermediate risk group. Cost effectiveness also becomes questionable with greater need for downstream investigation and greater overall cost of care especially in those with moderate to high risk of ACS.

Magnetic resonance imaging

In an Emergency setting, accurate early diagnosis of ACS along with efficacious institution of treatment is the main objective. As aforementioned, ECG and biomarkers are all helpful but may not be able to pick out early or equivocal ACS. Furthermore, these tests are presently unable to distinguish with certainty, ACS from other potential differentials, establish the extent of myocardial involvement, determine whether the damage is reversible, or even define the culprit artery with any reliability.

CMR offers high spatial resolution, accuracy and high reproducibility thereby allowing detailed volume and functional assessment, excellent tissue characterization in any tomographic plane and exceptional prognostic ability with late gadolinium enhancement (LGE) imaging (Figure 2). Radiation free examination also affords the CMR with the ability to incorporate extensive imaging protocols and repeated imaging necessary for both clinical and research imperatives.

Studies have already shown that CMR techniques such as myocardial function, perfusion imaging and LGE is able to provide a more accurate diagnosis of ACS compared with standard clinical assessment that includes ECG and biomarkers^[51]. The use of new imaging techniques such as T2-weighted sequences for oedema detection also increases its diagnostic performance^[52]. Moreover, unlike CT-based imaging, CMR utility can be extended to patients with intermediate to high risk for ACS but without ECG or biomarker evidence of MI^[53].

In essence, CMR represents a "one-stop-shop" for early and comprehensive assessment towards accurate and reliable diagnosis, risk stratification and prognostication of patients with ACS.

Standard magnetic resonance imaging techniques

Rest cine magnetic resonance imaging utilises steady-state free precession sequences to acquire a series of consecutive, breath-hold, long and short-axis slices (Figure 3). The excellent spatial resolution, coupled with the high contrast between blood and myocardium allows the en-

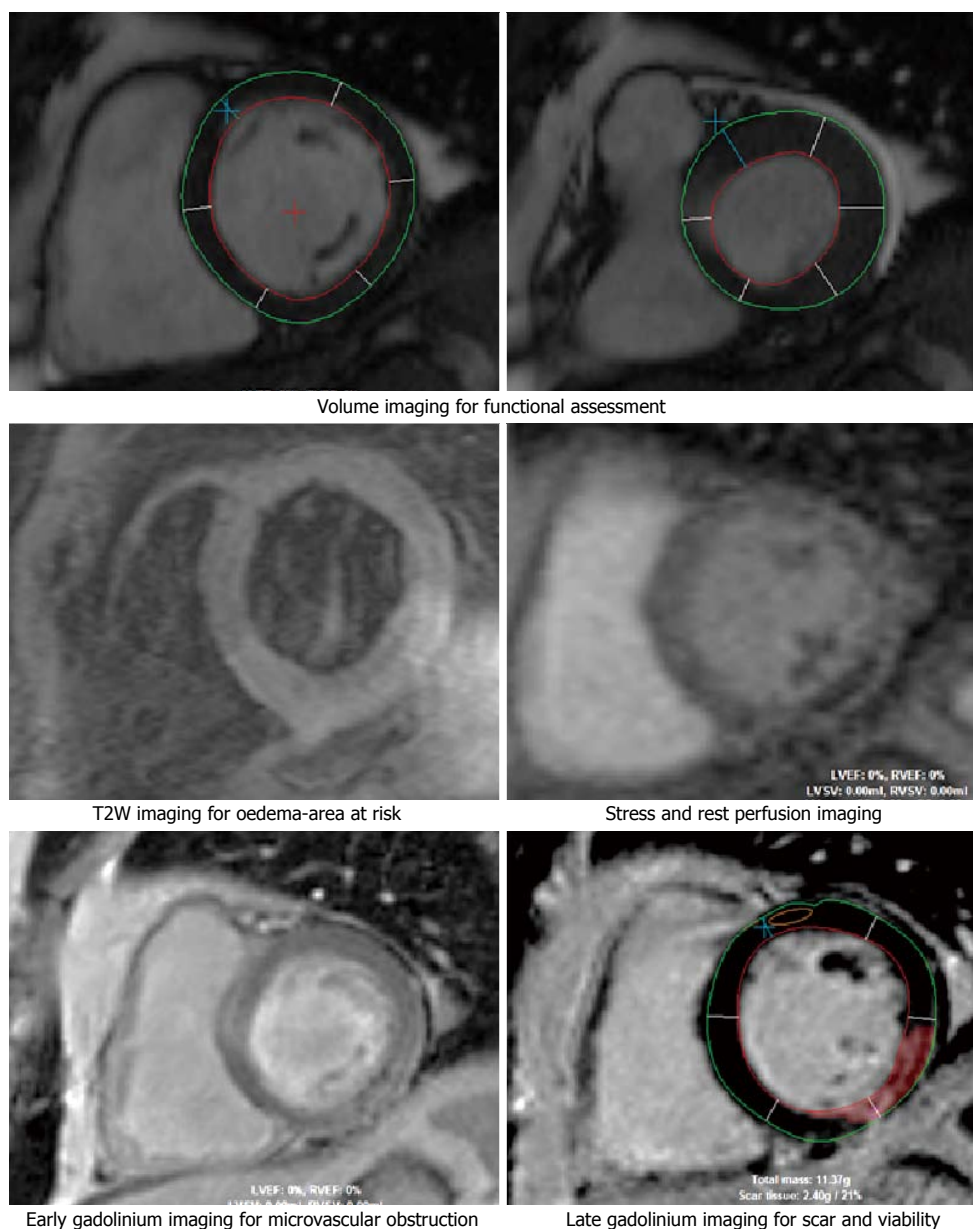


Figure 2 Cardiovascular magnetic resonance imaging sequence employed for the diagnosis of acute coronary syndrome. T2W: T2-weighted.

docardial border to be detected easily. This allows easy assessment of ventricular wall motion, ventricular volumes, ejection fraction, myocardial mass and anatomy of the extracardiac structures. These CMR assessments are accurate, reproducible and well validated^[54,55].

In the Emergency Department, these initial CMR imaging sequences can also be utilized to detect diseases of the aorta that may mimic ACS such as dissection or penetrating ulcer^[56]. Findings typical of myocarditis and Takotsubo cardiomyopathy can also be seen and confirmed by LGE^[57-60]. Initial review of the right ventricle and ventricular outflow tract, interventricular septum and pulmonary vasculature may also yield signs characteristic of acute pulmonary embolism which can then be subsequently confirmed with MR angiography^[61].

T2-weighted imaging

T2-weighted (T2W) imaging with short tau inversion re-

covery (STIR) sequences is used to detect myocardial oedema which has increased signal intensity. The presence of oedematous myocardial segments on T2W imaging is a sign of ischaemic myocardium and a negative prognostic indicator for cardiovascular events^[62]. Oedematous segments also allow acute-on-chronic differentiation of myocardial segments in established CAD patients^[63]. Acutely, T2W imaging also identifies the area-at-risk (AAR) which is defined as an area of potentially reversible myocardial injury but at risk of infarction. The extent of the AAR has been validated against histopathological and angiographic measurements and is predictive of the risk of further cardiovascular event or death^[62,64-66].

Perfusion imaging

Perfusion imaging is performed both at rest and stress (with Adenosine infusion) and assesses myocardial blood flow by capturing the transit of contrast medium through

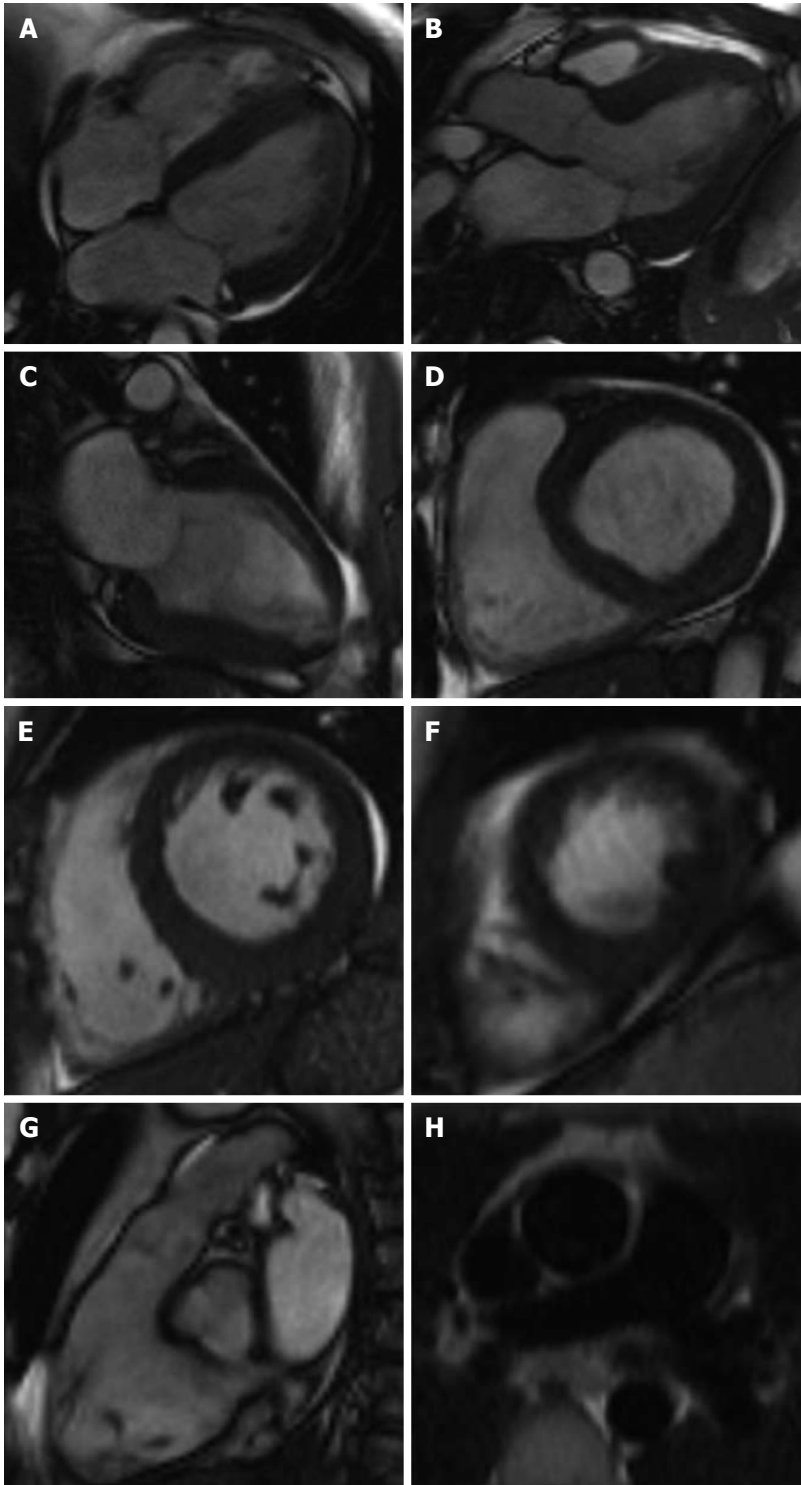


Figure 3 Standard imaging technique showing cine magnetic resonance imaging long axis views (A-C) and followed by short axis (D-F) and RVOT (G). Half-Fourier acquisition single-shot turbo spin-echo image shows the main, left and right pulmonary arteries (H).

the myocardium. It is a well established tool for assessing acute impairment in myocardial blood flow, patency of microvasculature, myocardial perfusion reserve and viability^[51,67]. In patients with chest pain with intermediate to high probability of ACS and a paucity of ischaemic signs, stress perfusion has a high negative predictive value with high diagnostic and prognostic value^[53,68].

CMR perfusion imaging is a potential alternative to

CT-based perfusion imaging due to improved subendocardial resolution, lack of ionizing radiation and cost effectiveness with reduced downstream investigation. Comparison with SPECT, PET and/or coronary angiography have shown good sensitivity and specificity of CMR in detecting perfusion defects of 87%-90% and 85%, respectively^[69,70]. Rest and stress perfusion imaging is well complemented by LGE and adds to a comprehen-

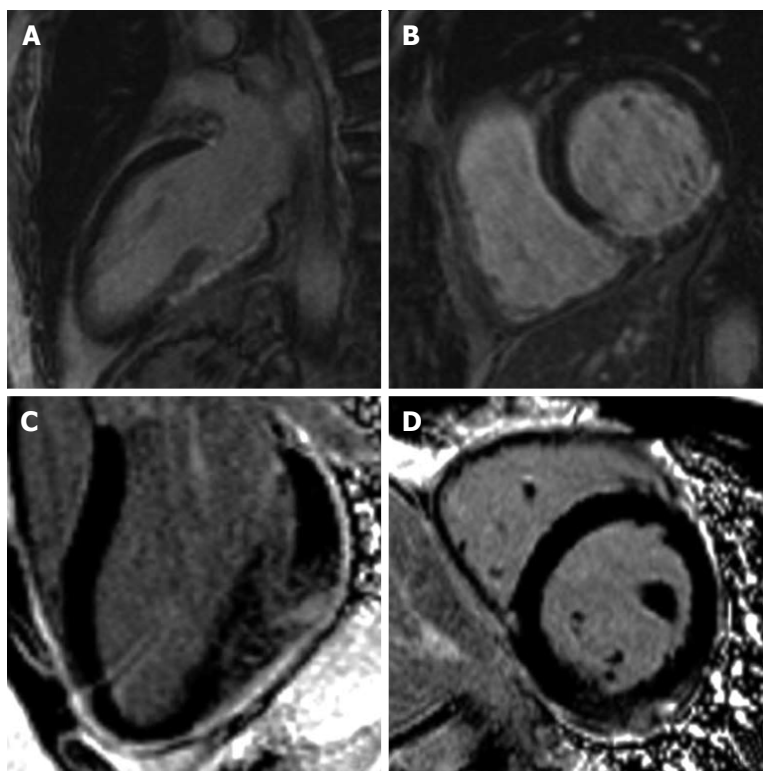


Figure 4 Late gadolinium enhancement showing two distinct hyperenhancement patterns. Subendo cardial for myocardial infarction (A and B) and epicardial for myocarditis (C and D).

sive assessment of patients with ACS. Its utility, reliability and accuracy in patients with intermediate to high risk of ACS also puts it ahead of CT-based perfusion studies.

LGE

Gadolinium based contrast is an extracellular contrast agent that accumulates in the interstitial space following myocardial death and replacement with fibrosis. Increased signal intensity denotes myocardial injury and scarring^[71]. Positive gadolinium enhancement coupled with CMRs high spatial resolution allows accurate and reliable quantification of the volume of injury and the transmural extent of the scarring^[72,73]. This is crucial in estimating the extent of the scar as a percentage of wall thickness with ramifications towards viability and therefore, reversibility of the underlying myocardial dysfunction^[74].

LGE essentially differentiates between irreversibly damaged (and thus non-viable) myocardium, from stunned myocardium which is ischaemic but viable. Acutely ischaemic but viable myocardium will have high signal intensity on T2W imaging but will be LGE negative. Generally, in a patient with MI, a transmural extent of scarring greater than 50% will signal a poor likelihood of functional recovery following revascularization^[75]. This has an important clinical ramification, as the prevalence of non-viable myocardial segments subtending the occluded epicardial artery will negate the need for immediate revascularization in an emergency setting.

LGE also has a role earlier in the diagnostic milieu of ACS by differentiating between ischaemic and non-

ischaemic causes of chest pain with biomarker rise. Differentials such as myocarditis and cardiomyopathy will have a different pattern of hyperenhancement. Ischaemia typically causes a more coalescent and subendocardial distribution of gadolinium enhancement confined to a particular vascular territory. Myocarditis has a typically epicardial or mid-myocardial distribution and cardiomyopathy has a patchy, mid-wall distribution (Figure 4).

LGE is also used in identifying microvascular obstruction (MVO) which is known angiographically as the “no reflow” phenomenon. Pathologically it is caused by failure of reperfusion at a microvascular level despite patent coronary arteries following revascularization. It is seen as a hypoenhanced core surrounded by hyperenhanced, scarred myocardium. MVO is well established as a negative prognostic marker and have been shown to be predictors of adverse remodeling following myocardial infarction^[76-78].

On another note, LGE is also of use for the detection of left ventricular (LV) thrombus which is a serious complication post-MI. It has a higher sensitivity and specificity than echocardiography for the detection of LV thrombus especially laminar, mural and apical thrombi^[79,80].

Prospect for clinical studies

CMR is already the gold-standard imaging modality for assessing left ventricular volumes, ventricular function and tissue characterization in cardiomyopathies. These factors along with infarct size and MVO are common surrogate end-points in many clinical trials and strong

predictors of clinical outcome. Other imaging sequences coming to the fore include T1 relaxation times with modified look-locker imaging, myocardial tagging and phase contrast imaging for flow assessment. These sequences are especially pertinent in assessing diastolic function which is becoming more routinely assessed and thus gaining greater importance in post-MI imaging.

Limitations of CMR

The main obstruction to incorporating CMR as a routine assessment for ACS in Emergency Department is the high capital outlay required both in terms of hardware and human resource. This limits the CMRs ability to accommodate emergency studies in an Emergency Department setting despite the manifold benefits that it offers. Likewise, newer imaging protocols introduced as part of clinical studies may lengthen the scan time beyond what is acceptable for revascularization targets and thus rule out its relevance in the Emergency setting. Having a strong magnetic field also negates its use in patients with metallic implants, aside from those who are claustrophobic. It is also not as mobile and easy to use as an echocardiogram and thus may not be usable in an intensive care unit setting for those who may gain the most from its use. More research is required into establishing the cost-effectiveness of CMR in routine clinical practice.

CONCLUSION

CMR allows comprehensive assessment of patients presenting to the Emergency department with chest pain. Its ability to accurately and reliably diagnose, risk stratify and prognosticate ACS puts it ahead of other imaging modalities currently available. Despite its manifold benefits, there are limitations to its wider use in routine clinical assessment and more studies are required into assessing its cost-effectiveness. It is hoped that with greater development in the technology and imaging protocols, CMR could be made less cumbersome, its imaging protocols less lengthy, the technology more inexpensive and easily applied in routine clinical practice.

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WJC 6th Anniversary Special Issues (5): Myocardial infarction

Clinical disease registries in acute myocardial infarction

Reza Ashrafi, Hussain Hussain, Robert Brisk, Leanne Boardman, Clive Weston

Reza Ashrafi, Department of Cardiology, Morriston Hospital, Swansea SA6 6NL Wales, United Kingdom

Hussain Hussain, Robert Brisk, Leanne Boardman, Clive Weston, Department of Cardiology, Singleton Hospital, Swansea SA2 8QA, Wales, United Kingdom

Clive Weston, College of Medicine, Swansea University, Swansea SA2 8PP, Wales, United Kingdom

Author contributions: Ashrafi R, Hussain H, Brisk R, Boardman L and Weston C all contributed equally to the paper with Weston C revising and editing the final submission.

Correspondence to: Dr. Clive Weston, MA, MB BCh, College of Medicine, Swansea University, Singleton Park, Swansea SA2 8PP, Wales, United Kingdom. c.f.m.weston@swansea.ac.uk

Telephone: +44-1792-513062 Fax: +44-1792-602846

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Core tip: Clinical disease registries are one of the oldest types of research methodology. They have been particularly important in the researching and guiding the management of myocardial infarction. Registries in multi-site studies can often be cheaper and simpler to undertake and less demanding of patients, and allow huge volumes of data to be collected from which many landmark studies already have been published.

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Abstract

Disease registries, containing systematic records of cases, have for nearly 100 years been valuable in exploring and understanding various aspects of cardiology. This is particularly true for myocardial infarction, where such registries have provided both epidemiological and clinical information that was not readily available from randomised controlled trials in highly-selected populations. Registries, whether mandated or voluntary, prospective or retrospective in their analysis, have at their core a common study population and common data definitions. In this review we highlight how registries have diversified to offer information on epidemiology, risk modelling, quality assurance/improvement and original research-through data mining, transnational comparisons and the facilitation of enrolment in, and follow-up during registry-based randomised clinical trials.

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Key words: Myocardial infarction; Acute coronary syn-

INTRODUCTION

Despite improvements in prognosis, myocardial infarction (MI) remains a major cause of death and morbidity^[1]. Significant structural, human and financial resources (nearly two billion euros/year in the United Kingdom) continue to be devoted to its management^[2]. This aspect of cardiological practice has been particularly well served by rigorous research using large randomised control trials (RCTs) of specific interventions or strategies-many of which have informed national and international guidelines^[3-5]. However, such guidelines are not automatically adopted. Clinicians may be slow to change, or uncertain where new findings fit into, their existing practice. They may fail to recognise, within a well-designed RCT, with its controlled environment, narrow inclusion criteria and intention to treat analyses, their own patient populations and complex (messy) working conditions, where what matters is not what treatment is “intended” but rather what is “given”, and the subsequent outcome. Registries

illuminate what is actually happening in practice.

Registries existed before the contemporary dominance of the RCT, and continue to flourish, as clinicians, researchers, healthcare companies, policymakers and patient advocacy groups recognise their importance. They complement the RCT, in as much as they allow an understanding of the extent to which the findings of RCTs are implemented in practice. Their analysis fills in some of the “gaps in evidence” concerning interventions for which RCTs have not been, or cannot be, performed or have not provided definitive answers. Additionally, they have a role in quality assurance, through clinical audit, and quality improvement initiatives and will play a central role in describing the outcomes of clinical care, from patient and payer perspectives.

There is no unified definition of a disease (or clinical) registry. While many registries fail to provide comprehensive outcome information the following two definitions highlight some of the key features:

“An organized system that uses observational study methods to collect uniform data (clinical and other) to evaluate specified outcomes for a population defined by a particular disease, condition or exposure, and that serves one or more predetermined scientific, clinical, or policy purposes”^[6].

“... a systematic collection of a clearly defined set of health and demographic data for patients with specific health characteristics, held in a central database for a pre-defined purpose”^[7].

So a registry is characterised by an intention to explore what is happening to patients with a particular condition or health need, pre-planning, explicit definitions of data items, a systematic approach to data collection, and a clear purpose.

In this anniversary edition, we review the historical background of registries, their characteristics, practical issues and future development in the management of myocardial infarction. Necessarily we will draw on our experience within the United Kingdom, but will also discuss other established national and international registries. We do not intend to provide an exhaustive catalogue of such registries, and mean no disrespect to colleagues whose registries we do not mention.

HISTORICAL DEVELOPMENT

The earliest registries were the personal records of individual physicians formed through their review of patient cases. These were presented and published as case series of particular conditions for the education of the wider medical community. The emphasis was on presentation and prognosis, rather than treatment of the recently recognised condition of coronary thrombosis. Examples of these early series, the precursors of the modern registry, can be found in the 1920s such as a seminal series of papers from Boston on MI and angina^[8]. By 1931, White and Bland^[9] were able to report on the prognosis of 200 cases of coronary thrombosis.

Collaborative, small scale, hospital registries began

to appear, normally containing observational reports on changing patterns of disease or outcomes of patients with MI^[10]. Interestingly, many of the most common clinical practices such as the use of the coronary care unit^[11] and description of Killip class^[12] were introduced following publication of analyses of disease registries.

In the late 1960s, there was great interest in a more collaborative international approach, to better understand the epidemiology of MI. The World Health Organisation (WHO) set up and co-ordinated a number of local MI registries (the MONICA project) which yielded much valuable information at a local level^[13]. This WHO initiative focussed on communities rather than hospitals, and was therefore able to capture information about those who died before reaching hospital and those who (as was common practice at that time) were managed at home by their general practitioners^[14]. Importantly, it promoted the collection of common datasets of information. The primary purpose remained “educational”-to more precisely describe the incidence of coronary events in a community, to categorise the various manifestations of heart attack and to compare fatality rates between communities. While others more recently have attempted to perform exhaustive community-based prospective studies of MI^[15,16] with an emphasis on expressing the “burden” of disease within a population-most existing registries are hospital-based (*i.e.*, patients are included/enrolled upon admission to hospital); the emphasis is on describing the provision of care and its effect on outcomes.

The need for a change in emphasis to allow such analysis was recognised by Hugh Tustall Pedoe in 1978 (echoing the thoughts of Osler, above): “The collection of information for its own sake is of doubtful value unless it is acted upon. Community registries should not become the equivalent of village war memorials”^[17].

He further stated that such information could be used in “monitoring the effects of treatment” and ensuring that it was “reaching those who needed it”. Here was an aspiration for registries to be used to assure provision of appropriate care and to record outcomes.

Long established, single-centre, registries (*e.g.*, the enduring Nottingham Heart Attack Registry, which began in 1972), instigated by interested clinicians rather than imposed by healthcare managers or professional bodies, provided fascinating insights into the changing management of MI^[18] but did not allow direct comparison with other units.

In some countries it was recognised that the administrative records generated to support well-developed insurance-based healthcare systems could be used for a secondary purpose: to create registries to compare care between hospitals (as “provider units”). In the United States, the Co-operative Cardiovascular Care project used billing information to investigate improvements in care, particularly for MI^[19]. The use of administrative data is now a common and cost-effective approach to data collection within registries.

More recently, there has been a general shift from registries as a mechanism for the “passive” reporting of

Table 1 Examples of key or historic exemplar registries of myocardial infarction

Registry/author	First publication year	Location	Setting	Key outcome
White ^[8]	1926	United States	Hospital	Prognosis of MI
Killip <i>et al</i> ^[12]	1967	United States	Hospital	Importance of coronary care unit
Tower Hamlets coronary project ^[14]	1972	United Kingdom	Community	Community based treatment and outcomes of MI
MONICA Project ^[23]	1987	Global	Various	Geographical variation, mortality and epidemiological trends
Second National Registry of Myocardial Infarction ^[24]	2000	United States	Hospital	Importance of door to balloon time in angioplasty
GRACE ^[25]	2002	Global	Hospital	Risk stratification in acute MI
EuroHeart Survey ^[26]	2002	European	Hospital	Quality improvement and assurance
MINAP ^[27]	2004	United Kingdom	Hospital	Epidemiology and quality improvement

MI: Myocardial infarction; MINAP: Myocardial Ischaemia National Audit Project; GRACE: Global Registry of Acute Coronary Events.

epidemiologic characteristics and the provision of treatments towards their use in an “active” process that assures and improves quality of care. Such an initiative can be appreciated in the Global Registry of Acute Coronary Events (GRACE)-a collaboration of over 100 volunteer hospitals in 14 countries to produce the largest multinational register of patients hospitalised with acute coronary syndrome^[20]. The development of this influential registry has been a milestone in the use of such data, not only in its “worldwide reach” but also in the underlying intention, to improve the care of MI.

Similar strategies of quality improvement and audit have been introduced in many countries. In the United Kingdom, the Myocardial Ischaemia National Audit Project (MINAP) began in 1998 with the intention to audit the management of all patients admitted to hospital in England and Wales following MI^[21]. The results of this project have allowed cardiologists to audit the performance of their hospital and focus on areas of inadequate performance in order to improve care^[22].

A selection of exemplar registries in MI through the years is shown in Table 1.

TYPES OF REGISTRY

MINAP is a (1) mandated, (2) continuous registry that uses a (3) unique data collection system to describe the (4) “whole-pathway” of care of acute coronary syndrome-from the onset of symptoms until discharge from hospital. It is designed to collect data on every case, regardless of where the patient is admitted within a hospital, though case ascertainment is incomplete. While some registries share these four attributes (*e.g.*, the Swedish SWEDE-Heart registry^[28]), others differ in this regard.

So, for example, in Italy the BLITZ programme consists four separate voluntary, time-limited, “snapshot” audits of care provided to a limited number of patients admitted to cardiac care units-the most recent being for a 10 wk period in 2010^[29]. In France the FAST-MI audit programme has, every five years since 1995, organised a month-long, nationwide, voluntary registry of consecutive patients admitted, with either STEMI or NSTEMI, to cardiac or intensive care units, within 48 h of symptom onset^[30]. The Acute Coronary Syndrome Israeli Survey is a biennial nationwide survey of acute coronary syndrome patients admitted to all 26 public hospitals in Israel dur-

ing a 2 mo period^[31]. An advantage of such intermittent (snapshot) data collection is the ability to collect very detailed and extensive data for a limited number of patients over a relatively short time (*e.g.*, 3079 patients over 1 mo in FAST MI 2010 compared with 79863 in the 12 mo from April 2010 in MINAP^[32]) without causing undue fatigue for data collectors. The long interval between snapshots allows adequate time for follow up of patients, for careful analysis of results and for the re-design of the next registry.

Some registries are designed to capture data for only certain sub-groups of patients with MI. So the ALERT-CZ registry reported on aspects of the pre-hospital treatment of patients admitted to 32 non-interventional hospitals in the Czech Republic^[33]; The Austrian Acute Percutaneous Coronary Intervention (PCI) Registry restricts analysis to those patients with acute coronary syndrome undergoing PCI, and so can provide accurate data on particular adjunctive drug treatments during such interventions^[34]; The Spanish EPICOR, a large registry sponsored by a pharmaceutical company, concentrates only on survivors of MI^[35].

As mentioned earlier, while many registries require active collection of data as an additional task, others use (or “mine”) routinely-collected administrative data, either as the sole data source, or, as in the case of MINAP, as the mechanism to provide basic follow-up information. Using administrative data restricts the types of question that can be answered through subsequent analysis, but considerably reduces the effort involved in collection. In many cases, at the local (hospital) level, there is no financial incentive to collect data and so anything that makes data collection less onerous is greatly advantageous.

Provision of data to registries may be voluntary on the part of the patient, such as the STENT registry on treatment of vein graft disease^[36], voluntary on the part of the hospital such as the Danish registry on mortality in ST-elevation and non-ST elevation MI^[37] or mandatory as part of a local legal or business framework-in some cases the successful completion of data is necessary if a hospital is to receive payment for the care provided.

FUNCTIONS OF REGISTRIES

Epidemiological information

Provision of epidemiological information-incidence and

prevalence, patient characteristics, intervention rates-the national Swiss AMIS Plus, and CZECH 1 and CZECH-2, being key examples of projects that can evaluate changes in epidemiology^[38,39].

Risk modelling and prognostication

Risk modelling and prognostication-as in the national MINAP registry^[40] and the multi-national GRACE-risk scores derived from such registries, and validated in others^[41], allow interventions to be targeted at those at highest risk, and therefore most likely to benefit, and, through use in case-mix adjustment, allow meaningful comparisons between hospitals and health systems.

Quality assurance

Quality assurance-registries can be used to measure performance against “best practice”, as described in national or international guidelines. In Europe, the first Euro Heart Survey on acute coronary syndromes was a large registry that looked prospectively at adherence to guidelines^[26] a second survey, repeated several years later, showed improved guideline adherence and superior outcomes^[42]. This has been confirmed in the Swedish registry where the adoption of evidence-based interventions (those shown to be beneficial in randomised trials) was shown to be associated with increased survival in those with STEMI^[43], and in MINAP where delivery of best and timely care (as expressed by a composite performance score) was associated with improved outcomes^[44].

Quality improvement

Quality improvement-registries can be designed, or opportunistically used, to monitor changes in process and outcomes of care, and so provide a good platform for assessing the effectiveness of quality improvement initiatives^[45]. Rather than being a passive tool to facilitate quality improvement, or a surrogate marker of a willingness to improve care (whereby voluntary participation in the registry is a sign of openness to change for the better), some have suggested that registries themselves provide the stimulus for instigating such initiatives. Major improvements in hospital performance and mortality rates have been reported following the public disclosure of hospital-specific results, with a substantial narrowing of the gap between the best and worst performing hospitals^[46].

Pursuit of research

Pursuit of research-while not their primary purpose, most registries lend themselves to the creation of generalizable knowledge^[47] and so to observational research. Such research, while adequate for hypothesis generation, for example the link between non-steroidal anti-inflammatory drugs and adverse cardiovascular events^[48], lacks the power to prove causality, but can be used to support findings from RCTs by reproducing the results of a trial in the large unselected captured in a registry population^[49]. However, analysis of registry data is complex, and often requires sophisticated multivariate analysis, sensitiv-

ity analysis and, because of incomplete data collection, imputation of missing values^[50] or propensity analysis^[51]. Notwithstanding these difficulties, the large volume of data held within a registry may be mined to yield important information. So, confirmation that earlier reopening of a coronary occlusion is beneficial was obtained not from a randomised trial of early *vs* delayed primary percutaneous intervention but from analysis of a registry that recorded door-to balloon times^[24]. Also, many registries have been used to show which pharmacological treatments are important in the MI population and how discontinuation can have significant negative outcomes for patients and have used this as a driver for improved post MI care^[22,52].

KEY PRACTICAL ISSUES IN REGISTRIES

With most registries there is a “trade-off”, or balance, between the richness of the data and data completion and case ascertainment rates. As the amount of information required for each case increases so do the demands placed upon local data collectors and, unless there is an explicit link between reimbursement for care and data collection, the likelihood that some cases will be included with incomplete data, and others will be missed altogether. This is of importance because there is evidence that those hospitals with poorer recording systems are also those with poorer outcomes^[53]. The extent of missing data is associated with 30-d mortality for STEMI and NSTEMI^[54]. This is less likely to be problematic in snapshot-type registries. Others have responded to this by introducing differing levels of participation, (*e.g.*, ACTION Registry-GWTG Premier and Limited levels of participation-the latter having 50% reduction in the amount of data collected^[55]) to allow centers that are experiencing particular problems with data entry to continue to register patients.

Some of the key properties of good registry design and performance and their practical aspects are shown in Table 2. A review of the advantages and disadvantages of the most common registry types is shown in Table 3.

ETHICAL AND GOVERNANCE ISSUES IN REGISTRIES

Data that can be collected from administrative records or medical case notes can be recorded without the individual patient’s knowledge or consent. Is this ethical? This is a point of significant controversy. Consideration of the principle of individual autonomy and right to personal privacy balanced against the greater good of future patients, as well as national statute, lead to significant variation in practice. Patients who refuse to give consent are systematically different from those who do not and their exclusion from registries is likely to skew findings^[59]. For this, and other reasons, some authors have argued that a regulatory insistence on individual choice is counterproductive, and that the standards suggested for

Table 2 Some key attributes of good registry design

Attributes of a good registry	Practical aspects
Standardised data collection and definitions	Pre-project agreement of common data definitions (<i>e.g.</i> , use of the Cardiology Audit and Registration Data standards ^[56]) and, where possible, standardised data collecting techniques
Rapid data collection	Computer web based data collection allowing rapid data accrual and transmission; agreed timeliness of data entry
Case ascertainment/ data completeness	Built in data checking during submission; regular data validation exercises (<i>e.g.</i> , the NCDR Data Quality Program ^[57]); comparison of case numbers with some other measure of unit activity; regular audit of participating sites to identify areas for improvement; explicit definition of participation in the registry and of a minimum dataset for each record; linkage to other complementary dataset ^[58]
Sequential enrolment	Allows for representative data without cherry-picking
Appointment of key stakeholders to a formal Steering Committee	Effective coordination of registry with oversight to share good practice and important results; guarantee analyses; clinical leadership and endorsement by professional bodies; regular revisions of the dataset reflect changes in practice
Random multi-site collection or mandated participation	Reduces the risk of population or site bias (as is common with RCTs in large academic city centres); enables comparisons between sites
Appropriate ethical considerations	Addresses both legal and ethical issues of patient consent; confidentiality; anonymity; data linkage (see below)
Clear and comprehensive result presentation	Clear and full results with meaningful and appropriate conclusions that reflect the findings and are presented in a way the target audience understands (<i>e.g.</i> , funnel plots); easy access to data and reports; clear explanations of any statistical adjustments
Transparent study background and funding	Prospective declarations of any issues

RCTs: Randomised control trials.

Table 3 Advantages and disadvantages of common registry types

Registry type	Benefits	Negatives
Academic	Limited external pressures for study; more flexibility in developing the dataset; lends itself to research; collaboration with many academic institutions and with Professional Bodies	Access to data provided by external sites may be limited; potentially limited funding; danger of “mission creep”-increasing data required; participating clinicians may become divorced from the academic group; difficult to enforce participation
Insurance	Ready access to data through billing information; large amounts of data held; potential for internal data linkage; large populations to study; excellent case ascertainment	Inability to expand dataset outside that determined by insurance company/HMO; difficult to influence/alter datafield definitions; full access to data may not be available due to commercial sensitivity
Industry sponsored	Well-funded; support for training of data collectors and encouragement of data entry; often based on access to new treatments	Limited sites; confidentiality clauses may restrict dissemination of findings; not all data widely available; may have strict patient selection (restricted to those receiving particular intervention); often time limited; less direct clinician control
Government	National “reach”; can promote and mandate high levels of participation and data collection; collaboration between multiple agencies; large population for study	Limited sense of clinical ownership

HMO: Health maintenance organization.

fully informed consent are too stringent and harm both research and public health^[60-62]. In the United Kingdom, the impact is low on patients whose data is included in a registry whose primary purpose is quality assurance and improvement and in which there is no intention to treat differently by virtue of participation, and so written consent is not required. The MINAP group, for example, has a legal exemption to hold patient-identifiable data without direct consent. As a result third party research access requires formal application of proposals to an academic steering committee and then only anonymised or pseudo-anonymised data is released after full academic review.

FUTURE DEVELOPMENTS

Registries will continue to develop beyond their original functions, becoming increasingly influential with respect

to quality improvement, regulation and research. This is predicated on an increased emphasis on professional accountability, the provision of safe, effective patient-centred care, and a shift of focus from the performance of particular interventions to the outcomes of the entire process of care. Increasingly, comparisons between clinicians, institutions and healthcare systems will be enabled through the implementation of common definitions for particular data fields across a range of registries. An international consortium of policy makers, clinicians, patient advocates and academics has identified registries as the mechanism through which to measure and report specific outcomes of the care of patients with coronary artery disease (including acute myocardial infarction) in a standardised way^[63], pointing to the need to share and to publicly report risk-adjusted data. Such transnational comparisons have recently been published following

painstaking analysis of two large national registries^[64]. Further, by understanding more about outcomes and costs of care it is hoped that patients will derive the maximum possible value of their interactions with clinicians in what has been called a “value-based” system^[65].

In addition to hard, readily/reliably measureable outcomes, such as death or length of stay in hospital, patients will be encouraged to report on their own outcomes following, and experiences of, care using a number of generic or disease-specific tools. These patient reported outcome measures or patient reported experience measures could potentially be gathered *via* integrated web services (with patient prompts), and provide a method of identifying important late complications which maybe outside the original data capture window^[66]. Furthermore, the social and emotional information contained within patient feedback may prove useful for the future design of services, and help understanding of adverse outcomes or difficulties in compliance with treatment.

If the ethical, legal and practical issues concerning the linkage of cases held in large datasets^[67] can be overcome, there will be further opportunities to appreciate the experiences and health needs of patients both before their index admission and thereafter. For example, the continuation of secondary preventive drugs following discharge from hospital with acute coronary syndrome has been assessed through linking the MINAP registry to a primary care dataset^[22]. It should be possible to link registries of heart attack to those of heart failure and cardiac rehabilitation, and so understand more fully the longer-term consequences of myocardial infarction.

Just as registries can provide information regarding the effects of quality improvement initiatives, so they can provide both a platform for enrolment and a mechanism for follow-up of patients participating in randomised trials of particular interventions; for example the TASTE trial of routine aspiration of intracoronary thrombus during primary percutaneous intervention^[68]. This technique, of registry-based randomised clinical trials, will significantly reduce the cost of interventional studies (to as little as 10% of the probable cost of an orthodox RCT in the case of TASTE) and maximise recruitment, while readily demonstrating the selective nature of the participating population through comparing the characteristics and outcomes of those enrolled with those excluded. The reduction in cost might also make possible important investigations of the utility of interventions for which there is no financial interest of the pharmaceutical or device industry—the usual sponsors of large trials—such as the role of supplemental oxygen in acute myocardial infarction^[69]. More investigator-initiated (either prospective/open-ended or time-limited/fixed-term) registries will be instigated to monitor the implementation of new technologies and to answer specific clinical questions^[70].

CONCLUSION

Registries have evolved greatly over the years from sources of epidemiological information to datasets whose analy-

sis can provide key information to clinicians, patients, researchers and medical policy makers. Registries will continue to provide important information on disease epidemiology, treatment and guideline adherence while being integral to quality improvement strategies in many disease states, as is already the case for MI.

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WJC 6th Anniversary Special Issues (5): Myocardial infarction

Timely reperfusion for ST-segment elevation myocardial infarction: Effect of direct transfer to primary angioplasty on time delays and clinical outcomes

Rodrigo Estévez-Loureiro, Ángela López-Sainz, Armando Pérez de Prado, Carlos Cuellas, Ramón Calviño Santos, Norberto Alonso-Orcajo, Jorge Salgado Fernández, Jose Manuel Vázquez-Rodríguez, Maria López-Benito, Felipe Fernández-Vázquez

Rodrigo Estévez-Loureiro, Armando Pérez de Prado, Carlos Cuellas, Norberto Alonso-Orcajo, Maria López-Benito, Felipe Fernández-Vázquez, Division of Cardiology, Complejo Asistencial Universitario de León (CAULE), 24071 Leon, Spain
Ángela López-Sainz, Ramón Calviño Santos, Jorge Salgado Fernández, Jose Manuel Vázquez-Rodríguez, Division of Cardiology, Complejo Hospitalario Universitario A Coruña (CH-UAC), 15006 La Coruña, Spain

Author contributions: López-Sainz A, Pérez de Prado A, Cuellas C and López-Benito M performed research; Calviño-Santos R, Salgado Fernández J and Vázquez-Rodríguez JM reviewed critically the literature; Estévez-Loureiro R wrote the paper; Alonso Orcajo N and Fernández-Vázquez F reviewed the text and gave final approval.

Correspondence to: Rodrigo Estévez-Loureiro, MD, PhD, Division of Cardiology, Complejo Asistencial Universitario de León (CAULE), Altos de Nava s/n, 24071 Leon, Spain. roiesteve@hotmmail.com

Telephone: +34-987-237400 Fax: +34-987-233322

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attractive way of diminishing delays. The purpose of this review is to address the effect of direct transfer on time delays and clinical events of patients with STEMI treated by PPCI.

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Key words: Primary angioplasty; Direct transfer; ST-segment elevation myocardial infarction network; Primary percutaneous coronary intervention; Myocardial infarction

Core tip: Primary angioplasty has emerged as the preferred reperfusion modality for patients with ST-segment elevation myocardial infarction. However, this treatment is associated with longer delays. Several strategies have been proposed to overcome these drawbacks. This review aimed to highlight the effect of a direct transfer strategy on time delays reduction and in the prognosis of this subgroup of patients.

Abstract

Primary percutaneous coronary intervention (PPCI) is the preferred reperfusion therapy for patients presenting with ST-segment elevation myocardial infarction (STEMI) when it can be performed expeditiously and by experienced operators. In spite of excellent clinical results this technique is associated with longer delays than thrombolysis and this fact may nullify the benefit of selecting this therapeutic option. Several strategies have been proposed to decrease the temporal delays to deliver PPCI. Among them, prehospital diagnosis and direct transfer to the cath lab, by-passing the emergency department of hospitals, has emerged as an

Estévez-Loureiro R, López-Sainz A, Pérez de Prado AP, Cuellas C, Calviño Santos R, Alonso-Orcajo N, Salgado Fernández J, Vázquez-Rodríguez JM, López-Benito M, Fernández-Vázquez F. Timely reperfusion for ST-segment elevation myocardial infarction: Effect of direct transfer to primary angioplasty on time delays and clinical outcomes. *World J Cardiol* 2014; 6(6): 424-433 Available from: <http://www.wjgnet.com/1949-8462/full/v6/i6/424.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i6.424>

INTRODUCTION

Primary percutaneous coronary intervention (PPCI) is the current preferred strategy to treat ST-segment eleva-

tion myocardial infarction (STEMI) when performed in a timely-fashion and by experienced operators. This technique has yielded superior results over thrombolytic therapy even when long transfer distances are accomplished^[1-6].

It has been demonstrated a relevant prognostic role of reperfusion delays in STEMI and both door-to-balloon and total ischemic time have been linked to increasing mortality^[7-9]. Current guidelines recommend that door-to-balloon delay must be inferior to 90-120 min^[10,11]. However, time delays to deliver PPCI are usually longer than recommended in practice guidelines^[12,13] and this may nullify the advantages of mechanical reperfusion over lysis^[14,15]. To overcome this problem, several strategies have been proposed^[16-18] and national efforts have been claimed to address all organizational issues either in United States or in Europe^[19,20].

Among these strategies, direct transfer from the field, bypassing the emergency department, to the catheterization laboratory has emerged as a safe and effective protocol for minimizing PPCI-related delays^[21-28]. We aimed to review the current evidence regarding the effect of DT on time delays and on clinical outcomes.

TIME EFFECT IN REPERFUSION THERAPY

Experimental models have clearly shown that there is a close relationship between the extension of myocardial necrosis and the time elapsed since the coronary artery occlusion^[29-31]. The myocardial damage extends as a “wavefront phenomenon” from the subendocardium to the subepicardium and the amount of muscle that can be saved by reperfusion is related to the time that flow can be restored^[32].

In the clinical setting, this relationship was evident in the first studies where a reperfusion method was tested: the thrombolytic therapy. The GISSI trial compared the use of streptokinase against placebo in patients with STEMI and less than 12 h from symptom onset. The overall results showed a net clinical benefit of the thrombolytic therapy^[33]. But when results were divided between time delay categories the significant benefit was observed only in those patients that received the lytic in the first 6 h since the start of the symptoms. This finding was subsequently confirmed in the fibrinolytic therapy trials’ analyses where all studies including > 1000 STEMI patients and randomized to thrombolytic or placebo were included^[34]. This metaanalysis showed that there was a linear relationship between the time to lytic therapy and the benefit in terms of mortality. The benefit was greater in the first hour (35 lives saved/1000 patients treated) and progressively decline every hour until 12 h since symptom onset. It was calculated that the loss of benefit of every hour of delay was 1.6 lives per every 1000 patients treated. No survival benefit was observed for those patients randomized after 12 h. However, this concept was challenged in a similar analysis but with more studies included by Boersma *et al.*^[35] (22 studies, every study with > 100 patients randomized). Authors showed that this

time-survival relationship with lytics was better represented by a non-linear regression curve. Survival benefit was maximal in the first two h and thereafter it suffered a steep decline, maintaining the benefit until 12 h of delay.

The relationship between time delays and mortality was as well observed in the setting of PPCI. To assess this association two time intervals have been defined: time to treatment (TTT, interval elapsed between symptom onset and mechanical reperfusion) and door-to-balloon (DTB, time from arrival to interventional hospital and mechanical reperfusion). Both time intervals have been linked to mortality in STEMI patients treated by PPCI. De Luca *et al.*^[9] showed that every 30 min of delay in the delivery of PPCI increased the mortality by 7.5%. Cannon *et al.*^[7] analyzing the data from the NRMI-2 registry demonstrated as well that the DTB interval was associated with an increasing mortality, above all when it was greater than 120 min. This fact was confirmed subsequently in a more contemporary analysis of the NRMI-3 and 4 registries, noting that a DTB interval > 90 min was associated with worse prognosis^[8]. However, several publications have addressed the issue that the time delay effect is related to the risk profile of the patients. In this sense, those patients exhibiting high risk features [anterior wall myocardial infarction (MI), previous MI, advanced Killip class, data of hemodynamic instability], those presenting very early after symptom onset (< 2-3 h) and those in cardiogenic shock, time delays play a key role in their prognosis. On the other hand, those patients of low risk or presenting late are less affected by the delays in reperfusion^[36-39].

The aforementioned data allowed the establishment in the practice guidelines the recommended time intervals to deliver PPCI: a DTB time of \leq 90-120 min^[10,40,41]. If mechanical reperfusion cannot be achieved in this time frame, then a selection of thrombolytic therapy might be advisable. However, with the growing evidence of PPCI being superior to lytic therapy in terms of mortality and cardiac events, this mode of reperfusion rapidly gained adoption in the medical community^[1]. Notwithstanding, it was rapidly pointed out that the widespread use of PPCI was translated into the fact that most of the patients received their reperfusion treatment out of the time schedule proposed by guidelines. In an analysis of the NRMI-4 registry, Nallamothu *et al.*^[12] showed that only 4.2% of patients treated by means of PPCI had a DTB time of less than 90 min. In a more recent analysis by Chakrabarti, including as well transferred patients from non-PPCI hospitals, only 9.9% of patients were into the boundaries of practice guidelines^[13]. In Europe, even with a more organized system, delays are as well longer than suggested. Moreover, the retardation induced by the system of care is an independent factor associated with worse prognosis^[42]. Several retrospective studies have tried to elucidate the exact delay with PPCI which will nullify the clinical benefit compared to thrombolysis^[14,15,43,44]. This time frame has varied from 60 to 120 min, but all studies have limitations inherent

Table 1 Effect of direct transfer on time delays *n* (%)

<i>n</i>	DTB	TTT	FP	Staff	Ref.
161 (DT 13)	87 vs 168	-	14%	Physician	[24]
658 (DT 25.2)	-	146 vs 191	-	Physician	[25]
401 (DT 59.9)	124 vs 154	-	-	Paramedics	[55]
301 (DT 35.8)	74 vs 116	150 vs 203	7%	Paramedics (teletransmission)	[28]
344 (DT 39.2)	69 vs 123	158 vs 230	-	Paramedics	[21]
1437 (DT 42.9)	83 vs 103	150 vs 200	-	Paramedics (teletransmission)	[26]
581 (DT 78)	69 vs 118	149 vs 219	-	Paramedics (computed algorithm)	[61]
1194 (DT 21)	102 vs 125	189 vs 259	4.7%	Physician	[27]
1859 (DT 23)	105 vs 122	185 vs 255	-	Physician	[67]

DT: Direct transfer; DTB: Door-to-balloon; FP: False positives; TTT: Time to treatment; PPCI: Primary percutaneous coronary intervention.

to post-hoc analysis and registries. Therefore, the exact delay assumable is still elusive and, moreover, it may depend on the risk profile of the individual patient^[43,45]. Given the evidence supporting the benefit of PPCI over thrombolysis (even though when the patient should be transferred from a non-PPCI facility^[6], and likely related to a more stable effect of reperfusion^[46,47]) most of efforts of national societies is to implement STEMI networks well organized and strategies in order to minimize delays for a timely PPCI^[19,20,48-50].

STRATEGIES TO REDUCE TIME DELAYS IN STEMI NETWORKS

Taking the previous information into account, several efforts have been claimed to reduce the delays involved in the delivery of PPCI and there have been conducted studies to address the strategies associated with the greater reductions in time delays performing PPCI. Most of these studies have been conducted in United States through surveys to hospitals across the country and through analysis of how top hospitals develop their programs of PPCI^[16,17,51-53]. The most comprehensive analysis of all studies published has been reported by Bradley *et al*^[18]. Authors conducted a survey in 365 hospitals of United States trying to identify the independent predictors of lower DTB time. In their results 6 strategies were significantly associated with faster door-to-balloon interval: (1) Having an emergency physician activating the catheterization laboratory; (2) Having a single call to a central page operator activate the laboratory; (3) Having the emergency department activate the catheterization laboratory while the patient is en route to the hospital; (4) Expecting staff to arrive in the catheterization laboratory within 20 min after being paged; (5) Having an attending cardiologist always on site; and (6) Having staff in the emergency department and the catheterization laboratory use real-time data feedback.

Interestingly, the use of prehospital electrocardiogram (ECG) was not associated with lower delays in the overall

population. However, this strategy was associated with significantly lower time intervals if the emergency medical system activated the cath lab team while the patient was on route to the hospital. Simply diagnosing STEMI in the prehospital setting, activate the interventional team and move the patient directly to the catheterization theater avoiding the emergency department or the coronary care unit is what we call direct transfer strategy (DT).

DT IN PPCI

In recent years there have been several studies that have investigated the association of DT for PPCI with shorter time delays in the delivery of reperfusion. The publications differ in their geographic location, method of ECG interpretation, distance between the reference point and the cath lab and the definitions of the different intervals analyzed^[21-26,28,54-63]. Furthermore, it is noteworthy that there is no randomized study on the subject and the evidence that we have rests on observational studies. That is why the results are heterogeneous and difficult to compare.

Role of direct transfer on time delays

The results of the main studies regarding the effect of DT on time delays in PPCI are summarized in Table 1.

The most relevant publications in terms of number of patients, methodology and results are those published by Le May *et al*^[21], Pedersen *et al*^[26], Dieker *et al*^[61] and our group^[27]. Le May *et al*^[21] analyzed the effect of DT in 344 patients with STEMI treated in the metropolitan area of Ottawa. The farthest distance to the PPCI hospital was 59.5 km. In this publication 39.2% of patients were directly transferred to the catheterization laboratory. Notably, for various reasons 2% received fibrinolytic therapy. DT significantly shortened the time delays, with median DTB of 69 min compared to 123 min in the standard admission. A significant reduction in total ischemic time (median 158 min vs 230 min, $P < 0.001$) was also observed. Ambulances were handled by paramedics. Pedersen *et al*^[26] analyzed their records of STEMI from 2005 to 2008 and included in the analysis 1437 patients of whom 42.9% were transferred directly to the catheterization laboratory. The study region covers a large population nucleus but investigators stress that the maximum transfer distance was 10 km and 90% within 60 min of the interventional hospital. For DTB interval definition the first medical contact instead of the arrival to the interventional hospital was selected. This is in accordance with the new recommendations for measuring these intervals when transferred patients from non-PCI facilities are included^[64]. Direct transfer patients consistently showed less delay compared to the conventional admission strategy in the DTB interval (median 83 min vs 103 min, $P < 0.001$) and in the TTT time (median 150 min vs 200 min, $P < 0.001$). Sixty-one percent of patients were in the range of DTB < 90 min recommended by the guidelines. In this study, ambulances were equipped with ECG tele-

transmission and were staffed by paramedics. Dieker *et al*^[61] analyzed 581 patients from a region of Holland with transport distance of 77 km. DT was associated with lower time delays and a higher proportion of patients in the recommended time frame of guidelines (82% *vs* 23%, $P < 0.001$). In the publication by our group^[27], we studied the role of DT in 1194 patients with STEMI who underwent PPCI at our regional STEMI program. From that group, 255 (21%) experienced DT from the field. It must be stressed that our network has its farthest point of reference located 154 km from the PPCI-hospital. Our data showed that DT was as well associated with lower DTB and total ischemic time compared to those referred through emergency department route. And this finding was consistent for both patients from the catchment area of the PPCI-hospital and for that from catchment areas of non-PPCI hospitals. Furthermore, the longer the distance to the PPCI the greater the time saved by this strategy. Our results confirm and expand the previous observations to those regions with a STEMI network involving large transfer distances.

Role of direct transfer on clinical outcomes

While it is clear that DT reduces temporal delays, it is still more debatable if this strategy is associated with better clinical outcomes. Since the overall ischemic time is diminished a greater myocardial salvage is expected and this should impact prognosis of patients. However, we must take into account that publications of DT are retrospective and observational and the association between DT and clinical events may be biased. Moreover, in the early publications of the topic DT was associated with a negative impact on survival. In the publication of So *et al*^[55] DT group showed significantly higher mortality (13.3% *vs* 5%, $P = 0.001$) despite having less delay to reperfusion. But, we should highlight that these figures of mortality are unadjusted and DT patients presented cardiogenic shock more frequently and had higher percentage of intracranial hemorrhage. And even in 2009 a systematic review of studies published to that date with 980 patients concluded that there was still insufficient evidence to confirm that DT improved prognosis^[65]. However, this meta-analysis did not include the most recent studies and pooled together trials where fibrinolytic therapy and primary angioplasty were used. These features may explain why this meta-analysis failed to show benefit of DT on prognosis.

However, the most contemporary researches have changed this tendency and consistently pointed to a net clinical benefit with this strategy. In the study by Steg *et al*^[57], avoiding the emergency department was associated with lower early mortality (4.9% *vs* 8.6%, $P = 0.01$), being the Emergency service use a factor associated with a worse prognosis (OR = 1.67). At one year there was still benefit in mortality in the direct admission group (11.5% *vs* 15.6%, $P < 0.05$). In a post-hoc analysis of the On-Time trial^[23] patients in the DT group had a significant improvement in ejection fraction, less ventricular dysfunction [left ventricular ejection fraction (LVEF) <

40%] and lower 30-d mortality (1% *vs* 3.2%), although this finding was not statistically significant ($P = 0.2$). However at 1 year, DT was associated with lower mortality (2.1% *vs* 5.6%, $P = 0.04$), being direct admission an independent predictor of better clinical outcome (OR = 0.3). Pedersen *et al*^[26] were the first researchers to report an independent clinical benefit of DT. Authors showed a significant reduction in the composite endpoint of death or non-fatal myocardial infarction at 1 year (HR = 0.67). On the other hand, this study present the limitation that the individual figures of the clinical variables were not provided and they found no decrease in both “end points” individually. In the ACTION registry^[62], a registry regarding the use of prehospital ECG, which included patients not undergoing PPCI, a trend towards lower adjusted hospital mortality in prehospital diagnosis group was as well observed (6.7% *vs* 9.5%, OR = 0.80, $P = 0.06$). Dieker *et al*^[61] observed a lower mortality in the group of DT (7% *vs* 13%, $P = 0.03$). However the mortality reported was unadjusted and DT group were younger, with less diabetes mellitus and lower percentage of previous myocardial infarctions. In a novel study by Le May *et al*^[66] DT strategy was analyzed in 1389 patients. Death at 180 days occurred in 5.0% of patients transferred directly from the field, and in 11.5% of patients transported from the field to a non-PPCI-capable hospital ($P < 0.0001$). After adjusting for baseline characteristics mortality remained lower among DT group (OR = 0.52, 95%CI: 0.31-0.88, $P = 0.01$).

The most exhaustive analysis of the effect of DT on mortality was carried out by our group in two separate reports analyzing short (30-d) and long-term mortality (after a median follow-up of 2.4 years)^[27,67]. In the first study, we analyzed the effect of DT on 30-d mortality in 1194 patients. Patients transported directly had lower 30-d mortality (2.7% *vs* 6.8%, $P = 0.017$). After adjustment in a multivariable logistic regression analysis, DT remained as an independent predictor for improved outcome (OR = 0.33, 95%CI: 0.12-0.92). Subsequently we reported the effect of DT in a larger cohort and with the longest follow-up in the literature. In a multivariable Cox regression model the DT strategy persisted as an independent variable associated with a better prognosis (HR = 0.71, 95%CI: 0.50-0.99) (Figure 1).

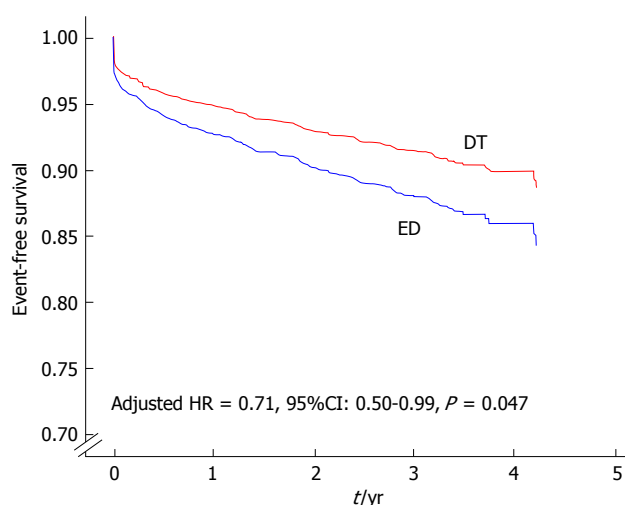
And finally, as we previously stated, the effect of time delays may be related to risk profile of patients. Therefore, the effect of DT on mortality might be influenced as well by some of the baseline characteristics. In this sense, Ortolani found a positive effect on survival of patients with cardiogenic shock who experienced direct transfer^[25,59] and our group found a suggested better outcomes in those patients with cardiogenic shock, diabetes mellitus, anterior wall myocardial infarction and those presenting ≤ 2 h from symptom onset (Figure 2)^[67]. The evidence of the effect of DT on clinical events is summarized in Table 2.

There are various reasons that may explain this survival benefit. First, patients have an earlier contact with

Table 2 Effect of direct transfer on clinical events *n* (%)

<i>n</i>	Short-term mortality	Late mortality	Adjusted mortality	Ref.
401 (DT 59.9)	13.3% vs 5%, <i>P</i> = 0.001	-	-	[55]
1204 (DT 66.9)	4.9% vs 8.6%, <i>P</i> = 0.01	11.5% vs 15.6%, <i>P</i> < 0.05	OR = 1.67 use ED	[57]
467 (DT 44.7)	1% vs 3.2%, <i>P</i> = NS	2.1% vs 5.6%, <i>P</i> = 0.04	OR = 0.3 if DT	[23]
344 (DT 39.2)	3.7% vs 5.7%, <i>P</i> = 0.3	6% vs 7.7%, <i>P</i> = 0.67	-	[21]
1437 (DT 42.9)	-	-	HR = 0.67 at 1 yr for death/reMI in DT	[26]
7098 (DT 27.4)	6.7% vs 9.5%, <i>P</i> = NS	-	OR = 0.80, <i>P</i> = NS in DT	[62]
581 (DT 78)	7% vs 13%, <i>P</i> = 0.03	-	-	[61]
1389 (DT 59.2)	3% vs 8.1%, <i>P</i> > 0.001	5% vs 11.5%, <i>P</i> < 0.001	OR = 0.52 at 180 d	[66]
1194 (DT 21)	2.7% vs 6.8%, <i>P</i> = 0.017	9% vs 16%, <i>P</i> = 0.005	OR = 0.33 at 30 d	[27]
1859 (DT 23)	3% vs 6%, <i>P</i> = 0.049	9.4% vs 14.4%, <i>P</i> = 0.008 at a median 2.4 yr	HR = 0.71 at 2.4 yr	[67]

DT: Direct transfer; ED: Emergency department; NS: Not significant.

**Figure 1** Cox regression survival curves. There is an adjusted survival benefit of direct transfer (DT). With permission, from reference [67]. ED: Emergency department.

the system, which provides a higher possibility of being in contact with staff that can deliver cardiac defibrillation and resuscitation if necessary, since it has been estimated that 50% of deaths occur in the prehospital phase^[68]. It is pertinent to recall that it has been observed consistently in the literature that the time from onset of symptoms to the contact with medical system is lower in DT group. It is possible that the DT group may represent a lower risk profile than those who come or are derived through emergency departments. They usually are younger, probably with clearer symptoms and possibly with a more definite ECG. This fact was shown previously^[69] and it is for this reason that when the effect of DT on mortality is assessed, it must be adjusted by this and other relevant variables. Despite this adjustment, the DT is still significantly associated with lower mortality. Second, it is clear that this strategy consistently reduces time delays and following the aphorism that “time is muscle” it is logical to find a prognostic benefit in these patients. The benefit of reperfusion regarding myocardial salvage is maximal in the first h of STEMI^[70] and this strategy allows greater diagnosis and treatment in the early stages, therefore driving to a more preserved LVEF. Moreover, the earlier treatment of patients with STEMI has been linked to a

better degree of “myocardial blush”^[71] and has also reported to significantly increase the percentage of thrombolysis in myocardial infarction 3 flow after PPCI^[61], both facts associated with improvement in LV function and prognosis. A recent publication has challenged the concept that lower DTB times are associated with lower in-hospital mortality^[72]. However, the retrospective nature of the study, the exclusion of transferred patients, the short DTB times and follow-up and the unadjustment for time from symptom onset to presentation may have affected the results. Since DT decreases all temporal delays in PPCI and not only DTB, we believe that this fact impacts positively the prognosis of patients. In addition, the prehospital diagnosis allows early initiation of antiplatelet and antithrombotic treatment. Drugs such as aspirin, clopidogrel, heparin and II b/IIIa inhibitors have been associated with an increased permeability of the infarct related artery preangioplasty^[73-76], a fact that has been associated with a better prognosis^[77].

CURRENT LIMITATIONS OF THE DT STRATEGY

This strategy, although in our view of enormous clinical benefit, has several limitations. First, it can only be applied in areas where a well-organized STEMI network is present. Second, despite having shown that the prehospital diagnosis by emergency medical system (EMS) ambulances and subsequent activation of the interventional cardiology team reduces delays, the use of these resources is underutilized. The main reason is probably related to the difficulty of general population awareness to call the EMS when there is a case of chest pain suggestive of myocardial infarction. Third, despite activating the EMS ambulances, not all of them have the capability to perform and transmit an ECG. In the ACTION registry^[62] done on more than 12000 patients, only 58.7% of the patients analyzed had contacted the EMS and only 27.4% had a prehospital ECG available. And together with the low frequency of prehospital ECG there is still the problem of its interpretation, raising the possibility of false activations of the cath lab with the consumption of unnecessary resources. In the literature false positive activations of the PPCI team with DT strategy have

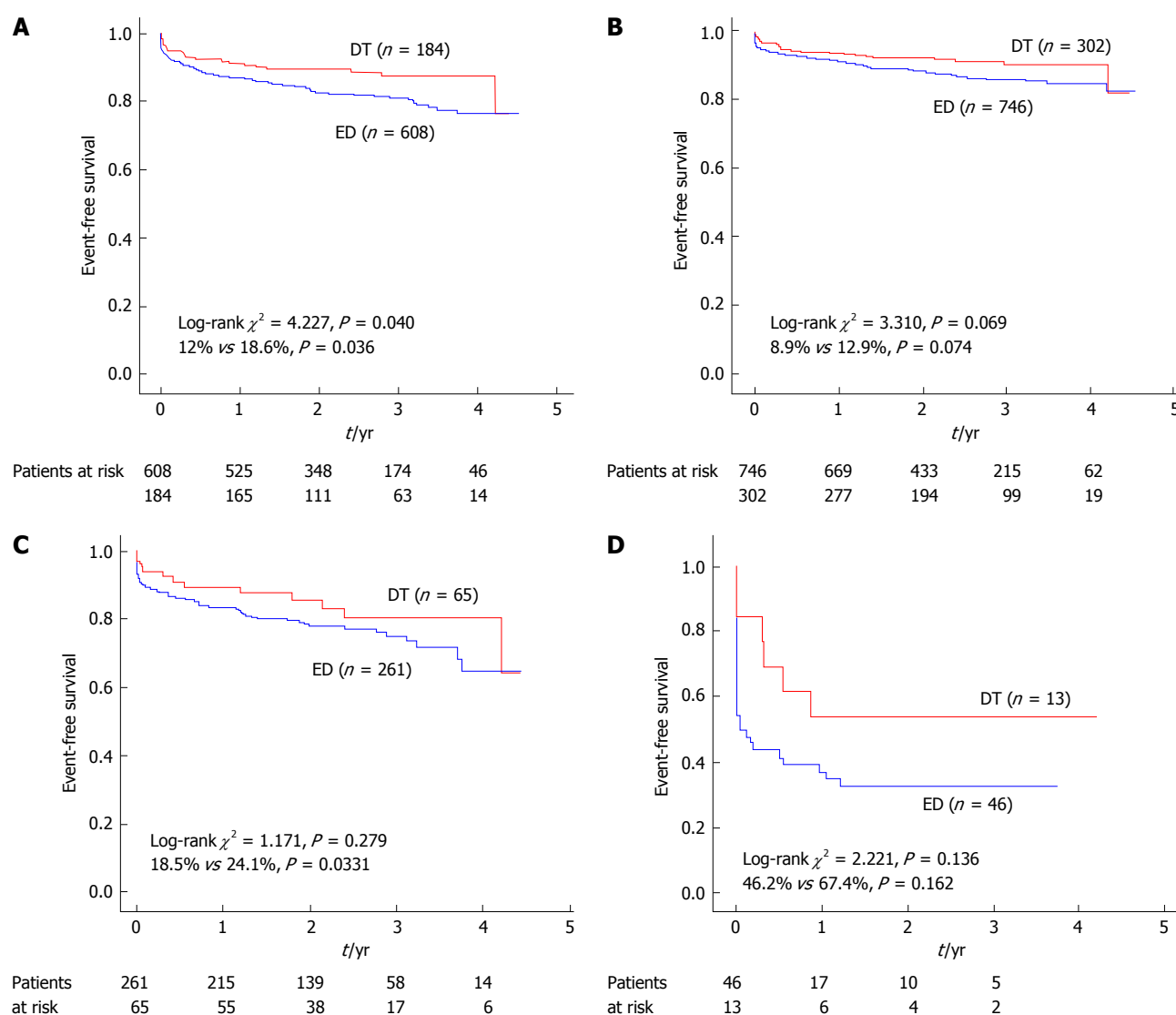


Figure 2 Kaplan-Meier survival curves for the different subgroups of higher risk. There is a trend to prognostic benefit in all subgroups that reaches significance in the group of anterior-wall myocardial infarction (MI). With permission, from reference [67]. A: Anterior wall MI; B: Early presenters; C: Diabetic patients; D: Cardiogenic shock. DT: Direct transfer; ED: Emergency department.

ranged from 0% to as high as 17%^[54]. However, when a STEMI network is well organized the false positive referrals from the EMS ambulances performing DT do not differ from those observed in the PPCI-hospitals and it is not associated with an increase in mortality^[78]. And finally, it is remarkable that, despite the benefits demonstrated and that is recommended in practice guidelines^[41], this strategy is underutilized in most of angioplasty networks. In a recent study in Canada^[79], and more than 15000 paramedics surveyed, only 18% (95%CI: 10%-25%) of EMS operators had protocols allowing the bypass of emergency departments in case of STEMI. We must work to increase the use of a technique that can offer prognostic benefits.

CONCLUSION

PPCI is the preferred reperfusion strategy in patients experiencing STEMI. On the other hand, it is associated

with longer time delays and most of patients do not meet the DTB limit recommended in practice guidelines. DT has emerged as a strategy that has consistently proved to reduce time delays and that is associated with an improved survival. However, it is still underutilized in most STEMI networks, so efforts must be done to increase the percentage of utilization.

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WJC 6th Anniversary Special Issues (5): Myocardial infarction

Novel adjunctive treatments of myocardial infarction

Michael Rahbek Schmidt, Kasper Pryds, Hans Erik Bøtker

Michael Rahbek Schmidt, Kasper Pryds, Hans Erik Bøtker,
Department of Cardiology, Aarhus University Hospital Skejby,
8200 Aarhus N, Denmark

Author contributions: Schmidt MR drafted the manuscript; Pryds K commented and revised the manuscript; Bøtker HK provided the idea for the manuscript, and completed manuscript revision and finalized the manuscript.

Correspondence to: Hans Erik Bøtker, MD, PhD, FESC, FACC, Professor, Department of Cardiology, Aarhus University Hospital Skejby, Brendstrupgaardsvej 100, 8200 Aarhus N, Denmark. heb@dadlnet.dk

Telephone: +45-78-452025 Fax: +45-78-452057

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Abstract

Myocardial infarction is a major cause of death and disability worldwide and myocardial infarct size is a major determinant of prognosis. Early and successful restoration of myocardial reperfusion following an ischemic event is the most effective strategy to reduce final infarct size and improve clinical outcome, but reperfusion may induce further myocardial damage itself. Development of adjunctive therapies to limit myocardial reperfusion injury beyond opening of the coronary artery gains increasing attention. A vast number of experimental studies have shown cardioprotective effects of ischemic and pharmacological conditioning, but despite decades of research, the translation into clinical effects has been challenging. Recently published clinical studies, however, prompt optimism as novel techniques allow for improved clinical applicability. Cyclosporine A, the GLP-1 analogue exenatide and rapid cooling by endovascular infusion of cold saline all reduce infarct size and may confer clinical benefit for patients admitted with acute myocardial infarcts. Equally promising, three follow-up studies of the effect of remote ischemic conditioning (RIC) show clinical prognostic benefit in patients undergoing coronary surgery and percutaneous coronary intervention. The discovery that RIC can

be performed noninvasively using a blood pressure cuff on the upper arm to induce brief episodes of limb ischemia and reperfusion has facilitated the translation of RIC into the clinical arena. This review focus on novel advances in adjunctive therapies in relation to acute and elective coronary procedures.

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Key words: Myocardial infarction; Primary percutaneous intervention; Coronary artery by-pass graft; Ischemia-reperfusion injury; Ischemic preconditioning; Remote ischemic conditioning; Cyclosporine; Cooling; Exenatide

Core tip: Patients with ischemic heart disease have a high risk of developing myocardial infarction, which is associated with considerable morbidity and mortality. Limiting the detrimental consequences of myocardial infarction is a major focus of cardiovascular research. Recent clinical studies suggest that novel adjunctive therapy with pharmacological and ischemic conditioning reduce ischemia-reperfusion injury in patients during coronary procedures. In three independent randomized trials, remote ischemic conditioning (RIC) improves clinical outcome in patients undergoing acute or elective percutaneous intervention or coronary artery by-pass surgery. RIC can be performed safely and non-invasively by intermittent inflation of a blood-pressure cuff on the upper arm and is easily applicable in most clinical settings.

Schmidt MR, Pryds K, Bøtker HE. Novel adjunctive treatments of myocardial infarction. *World J Cardiol* 2014; 6(6): 434-443 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i6/434.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i6.434>

INTRODUCTION

Heart disease and stroke are the leading causes of death

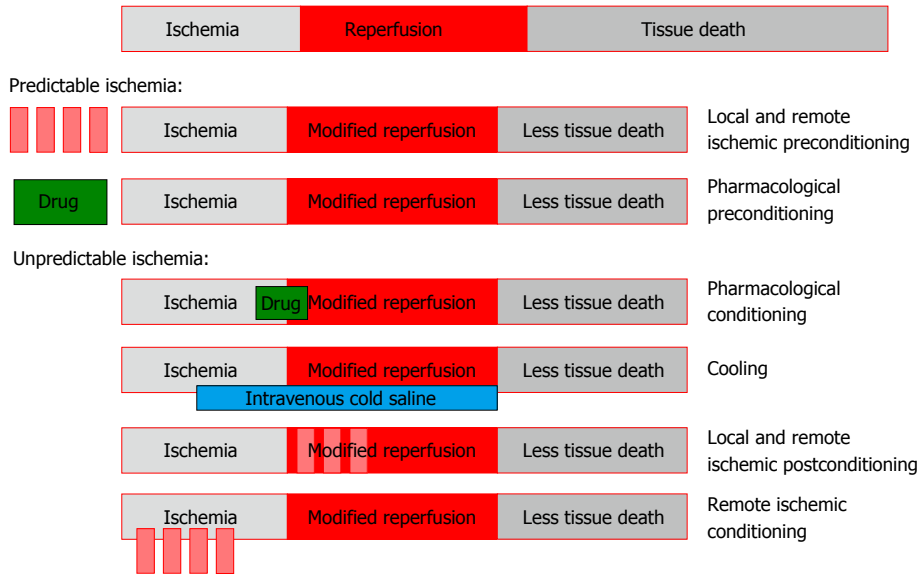


Figure 1 Overview of interventional strategies for achieving cardioprotection as adjunct to thrombolysis or primary percutaneous coronary intervention, see text for details.

worldwide^[1,2]. Since 1990, more people have died from coronary artery disease than any other death cause^[3,4].

In China, a staggering 230 million are estimated to suffer from cardiovascular disease, and three million Chinese die of cardiovascular disease annually, accounting for 41% of all deaths^[5,6]. In the United States alone, cardiovascular diseases, including ischemic heart disease and stroke, account for more than one-third deaths and an estimated 900000 heart attacks and 800000 strokes occur each year. In the remaining parts of the world, from the Sub-Saharan developing countries over booming South America to affluent areas in Europe and Asia, similar patterns are seen^[7,8].

Globally, socio-demographic factors, unhealthy life style, escalating obesity and suboptimal control of risk factors are likely to further aggravate the disease burden over the coming decades^[9]. In the Western world, nearly half of the male and a third of the female population will develop coronary artery disease^[10]. Partly driven by urbanization and adoption of Western life style, China undergoes a transition towards a similar health statistic^[11].

The pandemic of cardiovascular disease has immense negative effects on global population health and life expectancy. While attempts to modify risk factor and life style related growth in cardiovascular disease are important and have been successful in some parts of the world^[8], improved treatment of acute and chronic cardiovascular disease is also crucial to alleviate the disease burden.

This review will focus on novel advances in the treatment of coronary artery disease, particularly the recent reports of successful adjunctive therapy in relation to elective percutaneous coronary intervention (PCI), coronary artery by-pass graft surgery (CABG), and acute angioplasty (primary PCI) for ST-elevation myocardial infarction (STEMI).

PROTECTING THE HEART AGAINST ISCHEMIA-REPERFUSION INJURY

Ischemia-reperfusion injury is the essence of myocardial infarction in relation to acute coronary events, but ischemia-reperfusion injury also occurs during planned procedures such as elective PCI and CABG, although usually to a lesser extent. As the term implies, not just the ischemia itself but also the following reperfusion harms the myocardium. Although reperfusion ultimately saves the ischemic myocardium and it may seem paradoxical that reperfusion induces myocardial injury, several biological phenomena explain for this effect [for detailed reviews, please see Hausenloy *et al*^[12] (2013) and Heusch *et al*^[13] (2013)]. Of potential clinical importance, ischemic and pharmacological conditioning of the myocardium can modify reperfusion injury and significantly reduce the tissue damage (Figure 1).

LOCAL ISCHEMIC CONDITIONING

Local ischemic preconditioning, induced by brief periods of ischemia before a sustained ischemic insult, has long been known to afford potent protection against ischemia-reperfusion injury^[14]. However, the technique has inherent limitations as it requires interruption of blood flow to the target organ and, thus, can only be achieved in the operating room or during coronary angioplasty. Furthermore, additional time for the preconditioning procedure is required during surgery or during intervention. Preconditioning itself might cause deterioration of organ function or cause complications, such as emboli of atheroma, because of the intermittent aortic clamping or intermittent coronary balloon inflation. Hence, local ischemic preconditioning has not found widespread clinical use.

However, by instead applying the local ischemic con-

ditioning stimulus after the ischemic event (*e.g.*, at the time of reperfusion in primary PCI), so-called ischemic postconditioning, most of these obstacles for clinical use are overcome. In an experimental setting, ischemic postconditioning inhibits ischemia-reperfusion injury almost as efficiently as ischemic preconditioning^[15,16]. Some clinical studies suggest that local ischemic postconditioning reduces myocardial injury in patients undergoing primary PCI for acute myocardial infarction^[17,18], but another recently published trial did not confirm this effect^[19]. Furthermore, a large-scale trial of 700 patients admitted with STEMI randomized to either standard primary PCI or primary PCI followed by postconditioning, failed to show any effect on myocardial reperfusion and clinical endpoints^[20].

REMOTE ISCHEMIC CONDITIONING

Remote ischemic conditioning (RIC) by repeated short-lasting ischemia in a distant tissue—mostly achieved by intermittent interruption of circulation in a limb—has recently emerged as a promising adjunctive therapy to avoid organ damage, thereby improving the outcomes of well-established therapies.

From the site of the remote stimulus, through humoral^[21] and neuronal^[22,23] pathways, RIC activates several protective mechanisms in the target organ similar to those activated by local preconditioning. They include the reperfusion-injury salvage kinase^[24] and survivor activating factor enhancement^[25,26] signaling pathways. Furthermore, RIC modifies systemic inflammatory response^[27,28], prevents endothelial dysfunction^[29] and platelet activation^[30] following ischemia-reperfusion injury.

In experimental studies, RIC has been shown to afford protection against ischemia-reperfusion in the liver^[31], lung^[32], kidney^[33], brain^[34], and heart^[29].

The ability to induce organ protection by a simple, non-invasive stimulus has facilitated the translation of RIC into the clinical setting. In patients, RIC can be induced by 3–4 cycles of inflation (ischemia) and deflation (reperfusion) of a standard blood pressure cuff placed on a limb. Following the original description of the method in 1997^[35] and its translation to humans in 2002^[29], multiple randomized clinical trials have shown that RIC affords organ protection in many clinical ischemia-reperfusion syndromes, including the kidney^[33,36], brain^[37], and heart^[38–41].

COOLING

Moderate hypothermia induced prior to reperfusion reduces infarct size in animal models^[42–44]. A clinical pilot study has suggested that patients admitted with anterior STEMI who are rapidly cooled to a body temperature below 35 °C by the combination of cold saline infusion together with an endovascular cooling catheter before primary PCI develop smaller infarcts^[45]. However, difficulties in applying the technique in the clinical setting without

delaying treatment together with inconsistent results cause controversy about the clinical value and applicability^[46], although a recent pooled analysis of two clinical trials indicate a potential beneficial effect^[47]. Most recently, the CHILL-MI study, using a similar cooling technique as in the initial pilot study, showed that while cooling did not have a general cardioprotective effect, it seems to reduce infarct size in patients with anterior STEMI admitted for primary PCI within four hours of symptom onset. In addition, cooling caused a significant reduction in heart failure events^[48]. A possible explanation for an overall lack of cardioprotective effect in the CHILL-MI study may be the fact that cooling below 35 °C was only achieved in 76% of the patients, and that sufficient cooling may be crucial for achieving cardioprotective effects.

PHARMACOLOGICAL CONDITIONING

The increasing insight into the mechanistic pathways involved in local and remote ischemic conditioning has encouraged identification of potential targets for pharmacological intervention against ischemia-reperfusion injury. A vast number of pharmacological agents have been shown to afford cardioprotection in experimental models, including adenosine^[49], erythropoietin^[50], rotigaptide^[51], statins^[52], atrial natriuretic peptide^[53], glucose-insulin-potassium^[54], P-selectin antagonist^[55], cyclosporine^[56], exenatide^[57] and metoprolol^[58]. A larger number of these agents have been tested in clinical studies (Table 1) with ambiguous results, the most promising being cyclosporine^[64], exenatide^[67] and metoprolol^[75], all of which seem to consistently provide cardioprotection in the clinical setting. For a comprehensive review, please see Kloner (2013)^[76]. However, an important limitation—and a potential explanation for the lack of success of pharmacological conditioning with some drugs, is that most agents act through a single signaling pathway in the complex and interactive system of protective mechanisms activated by ischemic conditioning and cooling^[13].

Cyclosporine

Cyclosporine, a widely used immunosuppressant drug, is believed to facilitate its cardioprotective effects by inhibition of mitochondrial permeability transition pore opening, thus preventing mitochondrial destruction^[77]. In a study by Piot *et al.*^[64], administration of cyclosporine at the time of reperfusion in STEMI patients treated with primary PCI, was associated with a reduction in infarct size measured by creatine kinase and troponin I release. In a subgroup analysis, a similar reduction in infarct size was demonstrated on day 5 with cardiac magnetic resonance imaging (CMR). In a follow-up study, Mewton *et al.*^[78] found that this infarct-sparing effect of cyclosporine was persistent at 6 mo. However, in a more recent study, no effect was shown of early cyclosporine administration as an adjunct to thrombolysis in STEMI patients in relation to infarct size, left ventricular function, heart failure or death^[65].

Table 1 Clinical studies using pharmacological adjunctive therapy in patients with acute myocardial infarction

	Intervention	n	Outcome
Adenosine			
Mahaffey <i>et al</i> ^[59] , 1999 (AMISTAD)	Infusion of adenosine for 3 h as adjunct to thrombolysis	236	Reduction in infarct size
Kloner <i>et al</i> ^[60] , 2006 (AMISTAD II)	Infusion of adenosine for 3 h	2118	No difference in death or heart failure
Atrial natriuretic peptide			
Kitakaze <i>et al</i> ^[61] , 2007 (J-WIND)	Infusion of atrial natriuretic peptide for 3 d	569	Reduction in CK, increase in LVEF
Atorvastatin			
Kim <i>et al</i> ^[62] , 2010 (STATIN-STEMI)	Oral atorvastatin 80 mg before primary PCI	171	No difference in death, revascularization or infarct size
Hahn <i>et al</i> ^[63] , 2011	Oral atorvastatin 80 mg before primary PCI	173	No difference in infarct size
Cyclosporine A			
Piot <i>et al</i> ^[64] , 2008	Infusion of cyclosporine before primary PCI	58	Reduction in infarct size
Ghaffari <i>et al</i> ^[65] , 2013	Infusion of cyclosporine as adjunct to thrombolysis	101	No difference in infarct size, death, heart failure or LVEF
Erythropoietin			
Voors <i>et al</i> ^[66] , 2010	Single dose erythropoietin	529	No difference in LVEF or infarct size
Exenatide			
Lønborg <i>et al</i> ^[67] , 2012	Infusion of exenatide for 6 h	105	Reduction in infarct size
Bernink <i>et al</i> ^[68] , 2012 (EXAMI)	Loading dose of exenatide before PCI followed by infusion for 72 h	39	No difference in left ventricular function or infarct size
Woo <i>et al</i> ^[69] , 2013	Subcutaneously and intravenous exenatide before primary PCI followed by twice daily subcutaneous injection for 2 d	58	Reduction in infarct size and improvement of LVEF
Glucose-insulin-potassium			
Mehta <i>et al</i> ^[70] , 2005 (CREATE-ECLA)	Infusion of GIK for 24 h	20201	No difference in mortality
Selker <i>et al</i> ^[71] , 2012 (IMMEDIATE)	Out-of-hospital infusion of glucose-insulin-potassium	357	Reduced mortality among patients with cardiac arrest
δ-protein kinase C			
Bates <i>et al</i> ^[72] , 2008	2 doses of KAI-9803	154	No difference in infarct size
Lincoff <i>et al</i> , 2014	Infusion of delcaseritib for 24 h	1083	No difference in infarct size
P-selectin antagonist			
Mertens <i>et al</i> ^[73] , 2006 (PSALM)	Infusion of recombinant P-selectin glycoprotein ligand-immunoglobulin as adjunct to thrombolysis	88	No difference in ST-segment resolution or LVEF
Tardif <i>et al</i> ^[74] , 2013 (SELECT-ACS)	Infusion of inclacumab before PCI in NSTEMI patients	322	Reduction in troponin I and creatine kinase
Metoprolol			
Ibanez <i>et al</i> ^[75] , 2013 (METOCARD-CNIC)	Infusion of metoprolol before primary PCI	220	Reduction in infarct size and improvement of LVEF

LVEF: Left ventricular ejection fraction; PCI: Percutaneous coronary intervention; CK: Creatine kinase; NSTEMI: Non-ST-elevation myocardial infarction.

Exenatide

Exenatide, a glucagon-like peptide-1 analog, is primarily used as an anti-diabetic drug for patients with type 2 diabetes. However, in addition to its beneficial metabolic effect, exenatide is believed to induce cardioprotection through activation of ischemia-reperfusion injury survival pathways^[79]. Lønborg *et al*^[67] found that in STEMI patients, a 6 h infusion of exenatide started prior to primary PCI was associated with increased myocardial salvage measured by CMR. This increase in myocardial salvage from exenatide infusion translated to a reduction in final infarct size, although reserved for patients with short system delay (< 132 min from first medical contact to first balloon inflation)^[80]. In a recent study by Woo *et al*^[69], subcutaneous injection together with intravenous infusion of exenatide as adjunct to primary PCI followed by twice daily subcutaneous injections of exenatide for another two days, was associated with both a reduction in infarct size and improvement of left ventricular function.

Metoprolol

Most randomized clinical trials investigating potential infarct sparing effects of betablockers in STEMI patients have been conducted in the pre-reperfusion era, and only a few studies have evaluated the cardioprotective effect of beta-blockage as an adjunct to thrombolysis or primary PCI. However, recently, the METOCARD-CNIC trial demonstrated that intravenous metoprolol administered to STEMI patients prior to primary PCI was associated with significantly smaller infarct size measured by CMR compared to primary PCI treatment alone. In addition, early metoprolol administration increased left ventricular function^[75].

IMPROVING THE OUTCOME OF MYOCARDIAL INFARCTION IN PATIENTS

The translation of cardioprotective strategies to counter the detrimental consequences of ischemia-reperfusion in-

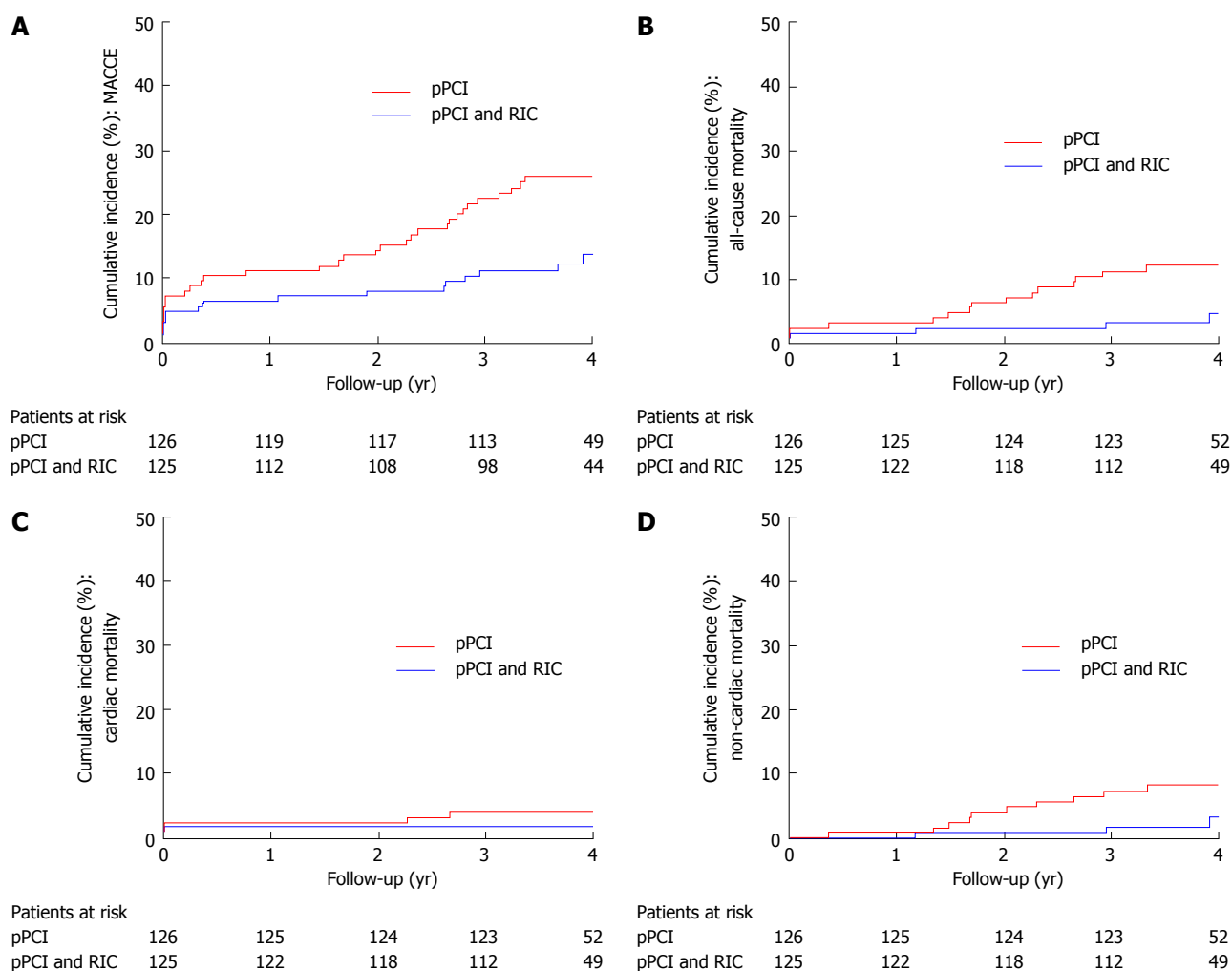


Figure 2 Effect of remote ischemic conditioning on long-term clinical outcome in patients with ST-elevation myocardial infarction treated with primary percutaneous coronary intervention. Cumulative incidence (%). A: Of major adverse cardiac and cerebrovascular events (MACCE) by year since randomization (per-protocol analysis). $P = 0.010$; B: Of all-cause mortality by year since randomization (per-protocol analysis). $P = 0.019$; C: Of cardiac mortality (%) by year since randomization (per-protocol analysis). $P = 0.248$; D: Of non-cardiac mortality (%) by year since randomization (per-protocol analysis). $P = 0.045$. Modified from Sloth *et al*^[89]. Eur Heart J (2014) 35: 168-175. pPCI: Primary percutaneous intervention; RIC: Remote ischemic conditioning.

jury is still in its infancy, and large-scale multicenter trials to show real-world clinical impact are lacking. However, recently published long-term clinical data on the use of RIC provide reason for optimism about a prognostic benefit of adjunctive therapy beyond opening the coronary artery.

REMOTE ISCHEMIC CONDITIONING IN PREDICTABLE ISCHEMIA

Predictable cardiac ischemia-reperfusion injury occurs in both elective PCI and CABG, and procedural tissue injury—as measured by biomarkers—is correlated to clinical outcome. Mid-scale clinical studies have shown that RIC applied prior to CABG^[39,81] and PCI^[38] reduces surrogate markers of myocardial injury, but until recently, the clinical relevance of these findings was questionable. However, two recent publications strongly suggest, that RIC should find a place as standard adjunctive therapy in elective PCI and CABG.

In a single center, randomized controlled trial, Davies

et al^[82] investigated the long-term clinical outcomes of 192 patients undergoing elective coronary angioplasty randomized to RIC or standard treatment. While their original study showed a significant reduction in troponin release in the RIC group^[38], the follow-up study revealed that this translated into a reduced major adverse cardiac and cerebrovascular events (MACCE) rate up to 6 years after the coronary intervention.

In another single center, double-blinded trial, Thielmann *et al*^[83] studied 329 low-risk patients undergoing elective isolated on-pump first-time CABG randomized to either standard CABG or CABG preceded by RIC. Besides reduced perioperative troponin I release as also shown previously by others^[84], the authors found a reduction in all-cause and cardiac mortality as well as MACCE in the intervention group during the follow-up period that was a mean of 1.5 year. During the follow-up period, MACCE occurred 23 times in the control group *vs* 8 times in the RIC group ($P = 0.011$). The authors observed 11 deaths in the control group and only 3 deaths in the RIC group ($P = 0.046$). The combined endpoint

(death, MACCE and repeat revascularization) yielded a HR of 0.38 (0.21-0.70) in favor of RIC. Interestingly, Thielmann *et al.*^[83] also found that RIC reduced the occurrence of sepsis, stroke and non-cardiac deaths, which adds to the speculation that RIC could confer systemic beneficial effects beyond the organ exposed to ischemia-reperfusion injury.

REMOTE ISCHEMIC CONDITIONING IN UNPREDICTABLE ISCHEMIA

In unpredictable ischemic events, like myocardial infarction and stroke, rapid restoration of blood flow to the ischemic territory has been the primary focus. Optimization of prehospital admission logistics to reduce any delay improves outcome^[85] and decreases mortality^[86]. While acute thrombolysis and primary PCI have improved outcome, recent studies show that further injury occurs early after reperfusion and can continue long afterwards^[87,88] emphasizing the need for therapies limiting clinical reperfusion injury in acute ischemic events.

In a study of 333 patients admitted with STEMI for primary PCI and randomized to either standard treatment or RIC performed in the ambulance during transportation to primary angioplasty, we showed, that RIC improves myocardial salvage index (0.75 in the RIC group *vs* 0.55 in the control group, $P = 0.033$) as measured by single-photon emission computed tomography^[40]. Recently, Sloth *et al.*^[89] published 4-year follow-up data on our original study, showing that the improved myocardial salvage translates into clinical prognostic benefit, as MACCE occurred for 17 (13.5%) patients in the RIC treated group compared with 32 (25.6%) patients in the control group, yielding a HR of 0.49 (95%CI: 0.27-0.89, $P = 0.018$). Furthermore, only 5 deaths (4%) occurred in the intervention group compared with 15 (12%) in the control group, yielding a HR of 0.32 (95%CI: 0.12-0.88, $P = 0.027$) (Figure 2)^[89]. Specific evaluation of death causes suggested a reduction in both cardiac and non-cardiac mortality, although only the latter was statistically significantly reduced (and most likely arose by chance). However, even when excluding non-cardiac deaths, MACCE was still reduced in the RIC group.

CONCLUSION

The globally increasing burden of cardiovascular disease calls for improved prevention and treatment. Acute and chronic coronary artery disease constitute the leading death cause in the World, and adjunctive therapies to limit the morbidity and mortality related to myocardial infarction may have major impact on global health. Pharmacological adjunctive therapy and rapid cooling decrease infarct size in some clinical studies but have yet to prove convincing clinical benefit. Remote ischemic conditioning-a low-cost, non-invasive and easily applicable adjunctive therapy-may confer prognostic benefit for patients undergoing coronary artery by-pass surgery and elective

and acute percutaneous coronary interventions. Large-scale studies with clinical endpoints, such as the ERICCA trial (ClinicalTrials.gov NCT01247545), the RIPHeart-study (ClinicalTrials.gov NCT01067703) and the CONDI 2 trial (ClinicalTrials.gov NCT01857414) are, however, needed to confirm the clinical effect, before RIC should be applied as standard adjunctive therapy. Similarly, as an adjunctive therapy to primary PCI the CIRCUS trial (ClinicalTrials.gov NCT01502774) will clarify the potential clinical benefit of cyclosporine A, and the DANAMI-3 trial (ClinicalTrials.gov NCT01435408) the potential clinical efficacy of ischemic postconditioning.

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WJC 6th Anniversary Special Issues (5): Myocardial infarction

Invasive strategy in patients with resuscitated cardiac arrest and ST elevation myocardial infarction

Vojka Gorjup, Marko Noc, Peter Radsel

Vojka Gorjup, Marko Noc, Peter Radsel, Department of Intensive Internal Medicine, University Medical Center Ljubljana, 1000 Ljubljana, Slovenia

Author contributions: Noc M designed figure; Radsel P designed table; all the authors wrote the article.

Correspondence to: Peter Radsel, MD, PhD, Department of Intensive Internal Medicine, University Medical Center Ljubljana, Zaloska 7, 1000 Ljubljana, Slovenia. peter.radsel@mf.uni-lj.si

Telephone: +38-61-5223182 Fax: +38-61-5222236

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Abstract

Coronary artery disease is the most frequent cause of sudden cardiac death. There is general consensus that immediate coronary angiography with percutaneous coronary intervention (PCI) should be performed in all conscious and unconscious patients with ST-elevation myocardial infarction in post-resuscitation electrocardiogram. In these patients acute coronary thrombotic lesion ("ACS" lesion) suitable for PCI is typically present in more than 90%. PCI in these patients is not only feasible and safe but highly effective and there is evidence of improved survival with good neurological outcome. PCI of the culprit lesion is the primary goal while PCI of stable obstructive lesions may be postponed unless post-resuscitation cardiogenic shock is present.

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Key words: Sudden cardiac arrest; ST-elevation myocardial infarction; Coronary angiography; Percutaneous coronary intervention

Core tip: There is general consensus that immediate coronary angiography with percutaneous coronary intervention (PCI) should be performed in all conscious and unconscious patients with ST-elevation myocardial

infarction in postresuscitation electrocardiogram. In these patients, acute coronary thrombotic lesion ("ACS" lesion) suitable for PCI is typically present in more than 90%. PCI in these patients is not only feasible and safe but highly effective and there is evidence of improved survival with good neurological outcome. PCI of the culprit lesion is the primary goal while PCI of stable obstructive lesions may be postponed unless postresuscitation cardiogenic shock is present.

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INTRODUCTION

Coronary artery disease has been documented in almost 80% of patients after resuscitated sudden cardiac arrest (CA)^[1,2]. In the past, most of these patients died either due to profound cardiac failure or post-resuscitation brain injury without any causative treatment^[3]. In year 2002 introduction of hypothermia, which was demonstrated to improve survival and neurological outcome of comatose patients, significantly changed the field of post-resuscitation treatment that became more intensive and cause-oriented^[4,5]. Besides, due to better pre-hospital "chain of survival" increasing numbers of patients after resuscitated cardiac arrest are being nowadays admitted^[6]. These include also patients with ST-elevation myocardial infarction (STEMI) in post-resuscitation electrocardiogram (ECG) requiring immediate coronary angiography (CAG) and percutaneous coronary intervention (PCI).

CAG

Despite the lack of randomized trials demonstrating ef-

Table 1 Non-randomized data on utilization of urgent coronary angiography and primary percutaneous coronary intervention in patients after resuscitated cardiac arrest^[1,7,9-48] *n* (%)

Author	Year	<i>n</i>	Comatose	STEMI	CA-PCI	PCI success	MIH	Survival	CPC 1 or 2	Survival comatose	CPC 1 or 2 comatose	Survival conscious
Kahn	1995	11	7 (64)	11/11 (100)	11 (100)	7/11 (64)	N	6/11 (55)	6/11 (55)	3/7 (43)	3/7 (43)	3/4 (75)
Spaulding	1997	84	NA	34/84 (40)	37 (44)	28/37 (76)	N	32/84 (38)	30/84 (36)	NA	NA	NA
Lin	1998	10	NA	10/10 (100)	10 (100)	10/10 (100)	N	9/10 (90)	NA	NA	NA	NA
Bulut	2000	10	NA	10/10 (100)	10 (100)	8/10 (80)	N	4/10 (40)	NA	NA	NA	NA
McCollough	2002	22	NA	22/22 (100)	22 (100)	22/22 (100)	N	9/22 (41)	NA	NA	NA	NA
Keelan	2003	15	13 (87)	15/15 (100)	15 (100)	14/15 (93)	N	11/15 (73)	9/15 (60)	NA	NA	NA
Bendz	2004	40	36 (90)	40/40 (100)	40 (100)	38/40 (95)	N	29/40 (73)	NA	NA	NA	NA
Quintero-Moran	2006	27	NA	27/27 (100)	27 (100)	23/27 (85)	NA	18/27 (67)	NA	NA	NA	NA
Sunde	2007	47	NA	NA	30 (64)	NA	Y	NA	NA	NA	NA	NA
Gorjup	2007	135	86 (64)	135 (100)	109 (81)	102/109 (94)	Y	90/135 (67)	74/135 (55)	44/86 (51)	25/86 (29)	49/49 (100)
Garot	2007	186	NA	186 (100)	186 (100)	161/186 (87)	Y	103/186 (70)	89/186 (48)	NA	NA	NA
Richling	2007	46	NA	46 (100)	46 (100)	NA	NA	24/46 (52)	22/46 (48)	NA	NA	NA
Markusohn	2007	25	18 (72)	25 (100)	25 (100)	22/25 (88)	Y	19/25 (76)	17/25 (68)	NA	NA	NA
Werling	2007	24	NA	NA	13 (54)	NA	NA	16/24 (67)	NA	NA	NA	NA
Hovdenes	2007	49	49 (100)	NA	36 (73)	NA	Y	41/49 (84)	34/49 (69)	41/49 (84)	34/49 (69)	NA
Valente	2008	31	31 (100)	31 (100)	31 (100)	NA	NA	23/31 (74)	NA	23/31 (74)	NA	NA
Mager	2008	21	NA	21 (100)	21 (100)	NA	NA	18/21 (86)	NA	NA	NA	NA
Wolfrum	2008	16	16 (100)	16 (100)	16 (100)	16/16 (100)	Y	12/16 (75)	NA	12/16 (75)	11/16 (69)	NA
Pleskot	2008	20	NA	NA	19 (95)	17/19 (89)	NA	NA	NA	NA	NA	NA
Peels	2008	44	NA	44 (100)	44 (100)	38/44 (86)	NA	22/44 (50)	NA	NA	NA	NA
Merchant	2008	30	NA	13 (43)	30 (20)	17/19 (89)	NA	22/30 (80)	NA	NA	NA	NA
Hosmane	2009	98	73 (74)	98 (100)	64 (65)	62/64 (97)	Y	63/98 (64)	57/98 (58)	39/73 (53)	33/73 (45)	24/25 (96)
Anyfantakis	2009	72	NA	23 (32)	27 (38)	24/27 (89)	NA	35/72 (49)	33/72 (46)	NA	NA	NA
Reynolds	2009	96	NA	42 (44)	NA	NA	Y	52/96 (54)	NA	NA	NA	NA
Lettieri	2009	99	NA	99 (100)	99 (100)	79/99 (80)	NA	77/99 (78)	72/99 (73)	NA	NA	NA
Pan	2010	49	NA	49 (100)	49 (100)	42/49 (86)	NA	31/49 (63)	NA	NA	NA	NA
Battista	2010	20	NA	10 (50)	20 (100)	NA	Y	8/20 (40)	6/20 (30)	NA	NA	NA
Dumas	2010	435	NA	134 (31)	202 (46)	177/202 (88)	Y	171/435 (39)	160/435 (37)	NA	NA	NA
Stub	2011	62	62 (100)	27 (44)	31 (50)	29/31 (94)	Y	NA	NA	NA	NA	NA
Tomte	2011	252	NA	NA	NA	NA	NA	140/252 (56)	108/212 (51)	113/171 (66)	73/171 (43)	41/41 (100)
Radsel	2011	212	171 (81)	158 (75)	165 (78)	150/165 (91)	Y	154/212 (73)	NA	NA	NA	NA
Mooney	2011	101	NA	68 (67)	56 (55)	NA	NA	NA	NA	NA	NA	NA
Cronier	2011	91	NA	50 (55)	46 (51)	43/46 (93)	Y	60/91 (66)	NA	NA	NA	NA
Moellmann	2011	65	NA	36 (55)	65 (100)	64/65 (98)	NA	46/65 (71)	NA	NA	NA	NA
Nanjayya	2012	35	35 (100)	31 (89)	21 (60)	NA	Y	20/35 (57)	14/35 (40)	20/35 (57)	14/35 (40)	NA
Bro-Jeppesen	2012	360	360 (100)	116 (32)	198 (55)	101/122 (83)	Y	219/360 (61)	207/360 (58)	219/360 (61)	207/360 (58)	NA
Zanutini	2012	93	93 (100)	32 (34)	NA	NA	Y	50/93 (54)	36/93 (39)	50/93 (54)	36/93 (39)	NA
Liu	2012	81	24 (30)	81 (100)	49 (60)	42/49 (86)	N	36/81 (44)	NA	NA	NA	NA
Zimmermann	2013	48	48 (100)	48 (100)	44 (92)	37/44 (84)	Y	32/48 (67)	16/48 (33)	32/48 (67)	16/48 (33)	NA
Hollenbeck	2013	269	269 (100)	0 (0)	122 (45)	NA	Y	151/269 (56)	NA	151/269 (56)	NA	NA
Velders	2013	224	108 (48)	224 (100)	217 (97)	NA	Y	183/218 (84)	168/218 (77)	NA	NA	NA
Skupaj	2013	3655	1499/1804 (83)	2012/3263 (62)	2253/3179 (71)	1373/1553 (88)	Y	2036/3384 (60)	1158/2241 (52)	747/1238 (60)	452/838 (54)	117/119 (98)

STEMI: ST-elevation myocardial infarction; PCI: Percutaneous coronary intervention; CA: Cardiac arrest; NA: Not available; MIH: Mild induced hypothermia

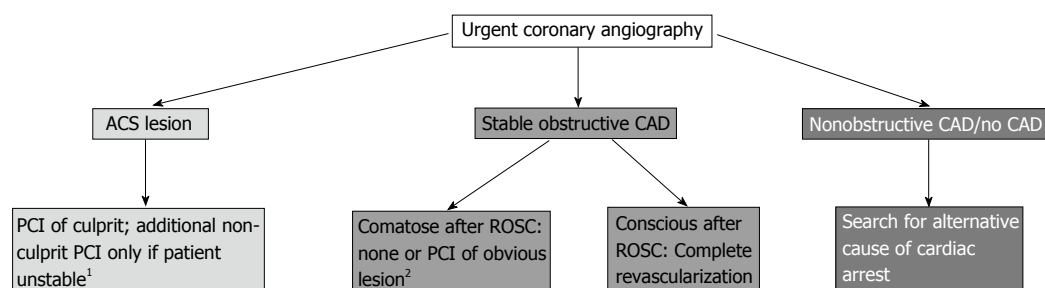


Figure 1 Revascularization strategy based on coronary angiography findings. ¹If ischemia or cardiogenic shock after successful culprit PCI; ²If considered responsible for cardiac arrest or beneficial for hemodynamic stability. ROSC: Return of spontaneous circulation; PCI: Percutaneous coronary intervention; CAD: Coronary artery disease.

fectiveness of immediate CAG and PCI in patients with resuscitated CA, we gradually increased the number of patients undergoing such immediate invasive coronary strategy. We extrapolated knowledge from randomized studies on acute coronary syndrome patients^[7] and generated our own experience on combination of immediate invasive coronary strategy mild induced hypothermia^[8,9]. After favorable experience with STEMI patients in post-resuscitation ECG, we applied the same protocol also to patients without STEMI in whom no obvious non-coronary cause of cardiac arrest was present. We were encouraged also by increasing number of independent peer-review experience by other investigators in more than 3500 patients cumulatively (Table 1). Patient selection and time to invasive procedure in these studies was different therefore results cannot be compared. Nevertheless we can appreciate that urgent PCI is feasible and highly effective in this population. There is also recent meta analysis of 10 observational studies showing immediate invasive coronary strategy to as independent predictor of survival (OR = 2.78; 95%CI: 1.89-4.10, $P < 0.001$)^[10].

Pubmed observational cohort studies on utilization of immediate CAG/PCI in patients with resuscitated sudden cardiac arrest (Table 1)^[1,8,11-49].

REPERFUSION STRATEGY

According to revascularization guidelines for STEMI without preceding CA^[50], CA-PCI should be primary directed towards “ACS lesions” for which we can assume direct cause-effect relationship with CA (Figure 1). The rationale is to reduce infarct size and improve hemodynamic and electrical stability. Patients who regain consciousness after return of spontaneous circulation have excellent prognosis (Table 1). Their survival is comparable or is even better that in general STEMI population without preceding CA. This may be partly explained by shorter ischemic times because of shorter patient delay. Index multi vessel and not only “culprit” PCI seems to be indicated only patients with post-resuscitation cardiogenic shock^[51]. We can speculate that complete revascularization improves left ventricular function, which may facilitate survival from post-resuscitation cardiogenic shock.

DISCUSSION

Nowadays, there is a question whether we should base our revascularization strategy for patients with STEMI in post-resuscitation ECG on non-randomized observational cohort studies. We believe, based on our experience and experience of others, that it would be very difficult to perform such prospective randomized trial. On the other hand we think such trial is needed for patients without STEMI in post-resuscitation ECG. However, regardless of this, we think patients with resuscitated cardiac arrest should be included in existing “STEMI networks” with direct transportation to the specialized “cardiac arrest centers” of excellence. Because of critical role of immediate CAG and PCI, interventional cardiologists should be an essential member of post-resuscitation team. However, when treating post CA patients we should avoid futility. In unfavorable settings of cardiac arrest (unwitnessed arrest, long delays to pre-hospital team arrival, no BLS, “non-shockable” first rhythm, long ACLS, recurrent arrest) or severe pre-arrest comorbidities, aggressive post-resuscitation treatment is not likely to result in quality survival.

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WJC 6th Anniversary Special Issues (5): Myocardial infarction

Impact of conditioning hyperglycemic on myocardial infarction rats: Cardiac cell survival factors

Christiane Malfitano, Alcione Lescano de Souza Junior, Maria Cláudia Irigoyen

Christiane Malfitano, Laboratório de Fisiologia Translacional, UNINOVE, 01504001 São Paulo, Brazil

Christiane Malfitano, Universidade Nove de Julho-Medicine Program, 01504001 São Paulo, Brazil

Christiane Malfitano, Maria Cláudia Irigoyen, Instituto do Coração, Universidade de São Paulo, Faculdade de Medicina, 05403900 São Paulo, Brazil

Alcione Lescano de Souza Junior, Nursing Department, University of Mato-Grosso, 78200000 Mato Grosso, Brazil

Author contributions: All authors contributed to this paper.

Correspondence to: Christiane Malfitano, PhD, Universidade Nove de Julho-Medicine Program, Rua Vergueiro 235/249-2° subsolo-Mestrado, 01504001 São Paulo, Brazil. chrismalfi@hotmail.com

Telephone: +55-11-33859241 Fax: +55-11-33859241

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Abstract

While clinical data have suggested that the diabetic heart is more susceptible to ischemic heart disease (IHD), animal data have so far pointed to a lower probability of IHD. Thus, the aim of this present review is to look at these conflicting results and discuss the protective mechanisms that conditioned hyperglycemia may confer to the heart against ischemic injury. Several mechanisms have been proposed to explain the cardioprotective action of high glucose exposure, namely, up-regulation of anti-apoptotic factor Bcl-2, inactivation of pro-apoptotic factor bad, and activation of pro-survival factors such as protein kinase B (Akt), vascular endothelial growth factor (VEGF), hypoxia inducible factor-1 α and protein kinase C- ϵ . Indeed, cytosolic increase in Ca²⁺ concentration, the mitochondrial permeability transition pore, plays a key role in the genesis of ischemic injury. Previous studies have shown that the diabetic heart decreased Na⁺/Ca²⁺ and Na⁺/H⁺ exchanger activity and as such it accumulates less Ca²⁺ in cardiomyocyte, thus preventing cardiac injury and the associated heart dysfunctions. In addition, the expression of VEGF

in diabetic animals leads to increased capillary density before myocardial infarction. Despite poor prognostic in the long-term, all these results suggest that diabetes mellitus and consequently hyperglycemia may indeed play a cardioprotective role against myocardial infarction in the short term.

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Key words: Conditioned hyperglycemia; Diabetes mellitus; Myocardial infarction; Cardioprotection; Survival factors

Core tip: Hyperglycemia or diabetes triggers a conditioned state that may protect the heart against ischemic injury and associated detrimental effects. These beneficial effects are present in short term diabetes and/or moderate hyperglycemia. The increase in glucose availability, the preferred energy substrate of the heart in stress condition, is likely to be one of the main cardioprotector mechanisms of hyperglycemia. However, other cardioprotective mechanisms seem to be involved, such as the release of cellular survival factors, ions preventing overload and angiogenesis. A fuller understanding of the mechanisms underlying conditioned hyperglycemia is then critical for the development of effective therapeutic strategies against ischemic heart disease.

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CONDITIONED HYPERGLYCEMIA AND MYOCARDIAL INFARCTION

Diabetes type 1 is a chronic disease characterized by hy-

perglycemia resulting from genetic and environmental factors. Complications of cardiac function are a leading cause of morbidity and mortality in type 1 diabetic patients^[1]. Diabetes induces cardiac dysfunction or diabetic cardiomyopathy, regardless of the presence or absence of vascular disease, coronary artery disease, arteriosclerosis and myocardial infarction^[2-4].

In hospital environments, glucose and insulin administration are induced in coronary artery bypass grafting patients. This therapy protects the myocardium and inhibits ischemia-induced adenosine monophosphate-activated protein kinase activation^[5]. However, intraoperative insulin resistance is associated with increased risk of complications, regardless of the patient's diabetic state^[6].

The increase in mortality in diabetic patients after myocardial infarction remains controversial. Intensive glucose control is widely used in patients with diabetes mellitus and stress-induced hyperglycemia. In this review study, we found that this strategy increases the risk of hypoglycemia, and dangerously increases catecholamine levels with hemodynamic response. Such significant changes may culminate in serious or even fatal cardiovascular events^[7].

Elevated admission glucose levels are common in patients with myocardial infarction and are strongly associated with increased mortality. Mortality of hyperglycemic patients was lower in the 1985 to 2008 period when compared to normoglycemic patients. Efforts to establish optimal treatment for these patients remain warranted^[8].

Accumulated evidence in clinical studies on diabetic cardiomyopathy suggests increased myocardial infarction and mortality in diabetic patients; however, experimental data regarding the increased resistance of diabetic animals to ischemic injury are quite controversial^[9]. Conversely, chronic hyperglycemia is associated with increased incidence of long-term cardiovascular complications, although its effect on acute hyperglycemic response and mortality after acute myocardial infarction remains unclear^[10].

One review study suggests that the diabetic heart may be more, equally, or even less susceptible to ischemia-reperfusion injury (novel cardioprotective strategy for the diabetic heart)^[11]. Our review study, however, aims at demonstrating the role of conditioned hyperglycemia as a protective mechanism of the heart after ischemic injury and in the preservation of cardiac function.

CELLULAR SURVIVAL FACTORS: CELL DEATH AND ANGIOGENESIS

Several studies have suggested that cardiomyocyte loss in ischemic cardiomyopathy may occur either by necrosis or by apoptosis, without significant inflammatory response^[12,13]. This loss has been found to contribute to the decline of the left ventricular function in humans^[14,15].

Indeed, experimental studies have shown that the chronic treatment of isolated cardiomyocytes with a high glucose content medium increased the rate of cell

death^[16]. In contrast, exposure to short periods of a high glucose medium or diabetes has been found to protect the heart against a variety of pathological insults, including ischemia, hypoxia, and calcium overload^[17-19]. Several mechanisms have been proposed to explain the cardioprotective role of high glucose exposure, such as up-regulation of antiapoptotic factor Bcl-2, inactivation of proapoptotic factor Bad, and activation of prosurvival factors^[17,20].

To investigate the mechanisms behind improved cardiac function (accompanied by a reduction in lesion area) in diabetic rats (30 d of hyperglycemia) undergoing myocardial infarction (15 d), we evaluated the gene expression regulating cardiac cellular survival factors: Bax, Fas, Bcl-2 and p53. In fact, gene expression was increased in diabetic animals after myocardial infarction, suggesting that the pro and anti apoptotic pathways can be activated simultaneously in this condition; this hypothesis was further strengthened by increased caspase-3 activity. These findings suggest an increased cell turnover acting to preserve cardiac function and reduce tissue injury^[21].

Cell survival factors can be activated by increased Bcl-2, as the up-regulation of Bcl-2 in some cells prevents excessive accumulation of calcium by mitochondria^[22], thus favoring cell survival. In this tissue, although calcium overload may be induced by ischemia, the association with hyperglycemia appears to reduce the activity of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger^[23].

Lending support to these findings, a study showed a reduction in protein expression of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger in diabetic infarcted hearts, which might contribute to mitochondrial disruption and contracture, inducing structural damage^[24]. In fact, the improvement in cardiac function in diabetic infarcted rats may be associated with the protective effect of Bcl-2, which abolishes the damage caused by the accumulation of calcium in the heart of diabetic rats.

Cytosolic Ca^{2+} overload during ischemia may be due to Ca^{2+} entry by reverse-mode of $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) secondary to the rise in Na^+ concentration. During ischemia, the anaerobic metabolism increases proton generation, which is extruded from the cell by Na^+/H^+ exchanger (NHE), resulting in increased cytosolic Na^+ concentration^[25]. This activates the reverse-mode of NCX exchanger, which in turn promotes an increase in Ca^{2+} concentration in the cardiomyocyte^[26]. Research has suggested that Na^+/H^+ exchange activity is decreased in diabetic hearts^[27]. Therefore, Ca^{2+} accumulation in the diabetic is lower than in the non-diabetic ischemic heart.

Several factors are related to cell survival: hypoxia inducible factor-1 α (HIF-1 α) is a transcription factor expressed in response to a decreased partial pressure of oxygen, and it is able to activate genes involved in angiogenesis, such as vascular endothelial growth factor (VEGF)^[28]. As a result of diabetic hyperglycemia, these survival factors were increased in diabetic animals before and after myocardial infarction^[21].

Interestingly, the expression of VEGF was also elevated before myocardial infarction in diabetic animals,

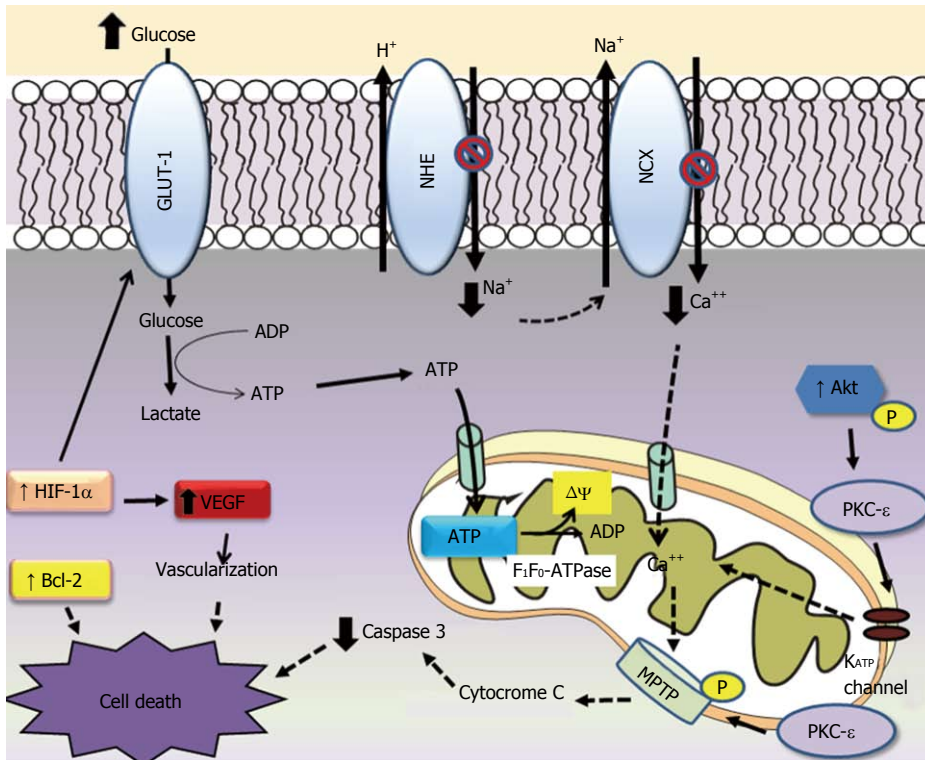


Figure 1 The process of apoptosis. GLUT-1: Glucose transporter type-1; NHE: Na^+/H^+ exchanger; NCX: $\text{Na}^+/\text{Ca}^{2+}$ exchanger; MPTP: Mitochondrial permeability transition pore; PKC- ϵ : Protein kinase C- ϵ ; VEGF: Vascular endothelial growth factor; HIF-1 α : Hypoxia inducible factor-1 α ; MPTP: Mitochondrial permeability transi-

and results were similar to the observed in interleukin 8 (IL-8) gene, *i.e.*, chemokine regulating neutrophil influx and activation with angiogenic propriety^[29-31]. IL-8 plays an important role in the recruitment of granulocytes in the infarcted myocardium, increasing cell adhesion (integrin) and activating the signaling pathways of cell survival mitogen-activated protein kinase and protein kinase C (PKC), which contribute to angiogenesis^[32]. Ooie *et al*^[33] have found that administration of streptozotocin for 12 wk in rats leads to increased tolerance to ischemic injury in an isolated heart model. These researchers also observed the translocation of protein kinase C- ϵ (PKC- ϵ) from the cytosol to the sarcolemmal membrane, where the protein is activated. PKC- ϵ is a K_{ATP} channel opener in both the sarcolemmal and mitochondrial membrane^[34]. Opening mitochondrial K_{ATP} channel during ischemia stabilizes mitochondrial potential, reduces mitochondrial Ca^{2+} overload, prevents ATP depletion, and the generation of reactive oxygen species^[34,35].

Mitochondrial permeability transition pore (MPTP) is a downstream of PKC- ϵ ^[36], which indicates that PKC interacts with MPTP, leading to phosphorylation of MPTP, and inhibits Ca^{2+} induced MPTP opening. Opening MPTP allows water and solutes to enter the mitochondria, increasing matrix volume and rupturing of the outer mitochondrial membrane. This results in the release of intermembrane cytochrome c, which can trigger apoptosis (Figure 1).

In this scenario, since hyperglycemia results in an increase of survival factors and induces angiogenesis, this may be interpreted as responses to repeated insults

which eventually determine an ischemic conditioning in diabetic rats. These responses are strongly associated with improved left ventricle (LV) function observed after ischemic injury, suggesting the presence of a physiological mechanism of protection against heart damage.

ROLE OF INFLAMMATORY CYTOKINES ON CARDIAC FUNCTION

Cardiac repair after myocardial infarction is dependent on the activation of tumor necrosis factor alpha (TNF- α), IL-1 β and IL-6 cytokines, which results in leukocyte recruitment to the infarcted area^[37]. In consequence, the immune imbalance between pro-inflammatory and anti-inflammatory properties can be modified in favor of more or less inflammatory factors, depending on the time course of the progression of heart failure. In this regard, changes in the concentration of TNF- α may have different effects on all the cell types involved in cardiac injury and repair, and in the suppression of cardiac contractility^[38] to improve cardiomyocyte apoptosis^[39].

In fact, Malfitano *et al*^[21] have found a reduction of TNF- α in diabetic rats after myocardial infarction. The signaling of IL-1 β is crucial for the activation of inflammatory and fibrogenic pathways in the healing of myocardial infarction, and it may play a role in the pathogenesis of post-infarction remodeling^[40]. Moreover, the induction of members of the IL-6 family leads to a rapid recruitment of mononuclear cells and cardiomyocyte ischemic myocardium^[41], thus indicating that the concentration of

IL-6 was increased only in infarcted rats, but remained unchanged in diabetic animals after ischemic injury.

These three pro-inflammatory cytokines are not only associated with the inflammatory response, but are also involved in heart failure, cardiomyopathy and LV remodeling, suggesting that the reduction of inflammatory factors may be one of the mechanisms responsible for improved heart function observed in this group. These findings corroborate a previous study of our group, in which it was demonstrated that hyperglycemia in mice and in cell culture is capable of suppressing the expression of pro-inflammatory mediators by apoptosis of neutrophils and lymphocytes^[42,43]. In fact, a high proportion of apoptotic lymphocytes in diabetic states strengthen the hypothesis that immune function is impaired in patients with poorly controlled diabetes^[42].

GLUCOSE METABOLISM IN CELL SURVIVAL

Another result which is in line with our findings is the increased expression of glucose transporter type-1 (GLUT-1) in diabetic rats after myocardial infarction. Indeed, previous studies have shown that the supply of glucose, with the regulation of GLUT-1, plays a critical role in cardioprotective response to myocardial ischemia^[21], with increased glucose supply during the acute ischemia^[44,45], and progression to heart failure^[46].

This is likely a result of increased availability and use of glucose, the preferred energy substrate of the heart in times of stress. Thus, the current clinical practice of tightly controlling blood glucose in patients having cardiac events may be detrimental to the heart in the acute setting^[47].

Much of the ATP generated by anaerobic glycolysis is consumed for the maintenance of ion gradient thought membranes. Part of the ATP generated is hydrolyzed by reverse mode of the mitochondrial F₁F₀-ATPase, which uses the energy to generate mitochondrial membrane potential ($\Delta\Psi$)^[48] (Figure 1).

CONCLUSION

Finally, the increase in survival pathways such as Bcl2, PKC- ϵ , Akt and in capillary density may effectively contribute to the reduction of ischemic injury and cardiac fibrosis (modulation of cardiac fibroblasts) in diabetic animals. This might be the key to a better heart function, as the increased GLUT-1 expression plays an important role in increasing glucose uptake in ischemic conditions. The clinical importance of the deficiency of glucose in the treatment of heart failure is not necessarily highlighted when blood glucose control is the pursued goal of treatment. In the DIGAMI II study reported on 1253 diabetic patients with acute myocardial infarction allocated to three treatment arms including acute insulin-glucose infusion followed by insulin-based long-term glucose control (group 1), insulin-glucose infusion followed by

standard glucose control (group 2), and routine metabolic management according to local practice (group 3) that neither all-cause mortality nor morbidity (stroke and non-fatal reinfarctions) differed between the three groups^[12].

The compensatory mechanism associated with the positive balance of regulatory genes related to program cell survival, reduction of inflammatory cytokines, and increased glucose use as energy substrate. Taken together, they promote greater plasticity and improved cellular resistance to ischemic injury in short term, suggesting an ischemic conditioning in hyperglycemia. These findings should be translated into more effective patient care strategies following ischemic events. Therefore, future studies should be conducted to further elucidate the mechanisms underlying conditioned hyperglycemia in cardioprotection after ischemia.

Possible cardioprotector mechanisms of conditioned hyperglycaemia or diabetes against ischemia and reperfusion injuries. Hyperglycaemia seems to be cardioprotective due to the increased glucose provision to heart during stress. In the ischaemia condition much of the ATP generated by glycolysis is breakdown by reverse mode of the mitochondrial F₁F₀-ATPase, which uses the energy to maintain mitochondrial potential ($\Delta\Psi$). Diabetic heart accumulates less Ca²⁺ due the inhibition NCX and NHE exchange activities. PKC- ϵ activity increases in diabetes, activating mitochondrial K_{ATP} channel and closing MPTP in the mitochondrial outer membrane. These effects reduce calcium overload, increasing ATP production and decreasing cytochrome C from mitochondria during ischemia. Hyperglycaemia increases anti apoptotic Bcl-2 protein and reduces caspase-3 activity. The contents of HIF-1 α mRNA and protein increase in diabetic heart. HIF-1 α target genes which in turn improve cellular oxygenation (VEGF) and glucose metabolism (GLUT-1).

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Metabolic, autonomic and immune markers for cardiovascular disease in posttraumatic stress disorder

Jeffrey L Kibler, Mischa Tursich, Mindy Ma, Lydia Malcolm, Rachel Greenbarg

Jeffrey L Kibler, Lydia Malcolm, Rachel Greenbarg, Center for Psychological Studies, Nova Southeastern University, Ft. Lauderdale, FL 33314, United States

Mischa Tursich, Department of Psychiatry, University of Western Ontario, London, Ontario N6A 5B7, Canada

Mindy Ma, Division of Social and Behavioral Sciences, Farquhar College of Arts and Sciences, Nova Southeastern University, Ft. Lauderdale, FL 33314, United States

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Correspondence to: Jeffrey L Kibler, PhD, Center for Psychological Studies, Nova Southeastern University, 3301 College Avenue, Ft. Lauderdale, FL 33314, United States. kibler@nova.edu

Telephone: +1-954-2625879 Fax: +1-954-2623857

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Abstract

Posttraumatic stress disorder (PTSD) has been associated with significantly greater incidence of heart disease. Numerous studies have indicated that health problems for individuals with PTSD occur earlier in life than in the general population. Multiple mechanistic pathways have been suggested to explain cardiovascular disease (CVD) risk in PTSD, including neurochemical, behavioral, and immunological changes. The present paper is a review of recent research that examines cardiovascular and immune risk profiles of individuals with PTSD. First, we address the relatively new evidence that the constellation of risk factors commonly experienced in PTSD fits the profile of metabolic syndrome. Next we examine the findings concerning hypertension/blood pressure in particular. The literature on sympathetic and parasympathetic responsivity in PTSD is reviewed. Last, we discuss recent findings concerning immune functioning in PTSD that may have a bearing on the high rates of CVD and other illnesses. Our primary goal is to synthesize

the existing literature by examining factors that overlap mechanistically to increase the risk of developing CVD in PTSD.

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Key words: Cardiovascular; Posttraumatic stress; Metabolic syndrome; Autonomic; Immune

Core tip: Research has documented a significantly increased cardiovascular disease (CVD) risk in posttraumatic stress disorder. The present paper is a review of recent research that examines cardiovascular and immune risk profiles of individuals with posttraumatic stress disorder (PTSD). First, we address the relatively new evidence that the risk factors commonly experienced in PTSD fit the profile of metabolic syndrome. Next we examine the findings concerning hypertension/blood pressure in particular. The literature on sympathetic and parasympathetic responsivity in PTSD is reviewed. Last, we discuss recent findings concerning immune functioning in PTSD that may have a bearing on the high rates of CVD and other illnesses.

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METABOLIC, AUTONOMIC AND IMMUNE MARKERS FOR CARDIOVASCULAR DISEASE IN POSTTRAUMATIC STRESS DISORDER

Posttraumatic stress disorder (PTSD), a disorder of

extreme stress/anxiety responses to a psychologically traumatic experience, has been associated with significantly greater incidence of heart disease^[1-4]. This effect has been demonstrated among combat Veterans^[1,5,6], firefighters^[7], and civilians^[2]. The characteristics associated with PTSD include re-experiencing symptoms such as intrusive thoughts and nightmares, avoidance behaviors, and arousal symptoms such as anger and hyper-vigilance. Lifetime prevalence of PTSD is about 8%, with higher rates among trauma victims and women. Numerous studies have indicated that health problems for individuals with PTSD occur earlier in life than in the general population^[6,8,9]. Further, there is limited evidence that the relationship of PTSD to physical health is independent of age, depression, or other comorbid anxiety disorders^[10]. Adult health problems may also be related to childhood trauma. In two large epidemiological studies, relationships were observed between childhood trauma and cardiovascular disease (CVD) evidenced as adults^[11,12], with up to 3 times greater risk of CVD. Multiple mechanistic pathways have been suggested to explain CVD risk in PTSD, including neurochemical^[13,14], metabolic^[15-17], and immunological changes^[18-24].

The present paper is a review of recent research that examines cardiovascular and immune risk profiles of individuals with PTSD. First, we address the relatively new evidence that the constellation of risk factors commonly experienced in PTSD fits the profile of metabolic syndrome^[25-28]. Next we examine the findings concerning hypertension/blood pressure (BP) in particular^[29-31]. The literature on sympathetic and parasympathetic responsivity in PTSD is reviewed. Last, we discuss recent findings concerning immune functioning in PTSD that may have a bearing on the high rates of CVD and other illnesses. Our primary goal is to synthesize the existing literature by examining factors that overlap mechanistically to increase the risk of developing CVD in PTSD.

METABOLIC SYNDROME AND PTSD

Most studies that have examined CVD risk factors in PTSD have not examined more than 1 or 2 risk variables, such as obesity or lipids. A study of police officers^[27] reinforced the importance of studying multiple CVD risk factors—this study revealed that those with the highest levels of PTSD symptoms (severe category) were 3 times more likely to exhibit 3 or more metabolic syndrome criteria [waist circumference, BP, high-density lipoprotein cholesterol, triglycerides, and glucose levels] than officers in the lowest PTSD symptom category (subclinical)].

The Violanti *et al.*^[27] findings are consistent with a recent study indicating Gulf War Veterans with higher severity of PTSD (measured on a continuum using the Clinician Administered Posttraumatic Stress Scale) were more likely to meet 3 or more of the CVD risk criteria for defining metabolic syndrome^[26]. Further analyses of these data by Heppner *et al.*^[32] indicated that antipsychotic medication use did not explain the increased risk for met-

abolic syndrome in severe PTSD. Similarly, among 245 low-socioeconomic-status subjects from general medical clinics in an inner-city hospital, significantly higher rates of metabolic syndrome were identified among patients with current PTSD, independent of antipsychotic medication use^[28].

Subsequent studies added to the literature providing evidence for the association of PTSD with metabolic syndrome. In one study, the prevalence of metabolic syndrome and its components were compared between patients with chronic war-related PTSD in Bosnia and Herzegovina vs patients without PTSD who underwent treatment for somatic problems^[33]. A significantly higher rate of metabolic syndrome was evident in patients with PTSD relative to the patients without PTSD, with hyperglycemia and abdominal obesity being more prevalent in patients with PTSD^[33]. Additionally, in a large retrospective database study of 207954 veterans^[25], metabolic syndrome was significantly higher in PTSD as compared to non-PTSD individuals. The results suggest PTSD accounted for 41% of the risk for metabolic syndrome^[25].

BLOOD PRESSURE AND PTSD

Early studies revealed elevated BP among combat veterans with PTSD^[34-36]. However, recent studies and meta-analytic reviews have reflected mixed findings^[29,30,37,38], raising doubt about the extent to which elevations in BP are consistently related to PTSD and might be a factor in CVD risk. Results of the meta-analyses by Buckley *et al.*^[29] and Pole^[30] suggested elevations in both resting systolic blood pressure (SBP) and resting diastolic blood pressure (DBP) for individuals with PTSD, when examining unweighted effect sizes. However, examination of weighted effect sizes produced much more circumscribed findings for BP in PTSD; the weighted effect sizes appeared to be conservative adjustments, as the mean effect sizes were reduced considerably relative to the unweighted means. In these meta-analyses, most studies of resting BP were fairly homogenous in terms of sample size, with only one study having a sample size greater than 115 ($n = 991$ for Keane *et al.*^[39]). This one very large study, which is weighted heavily for the meta-analyses, resulted in null effects for resting SBP and DBP. A potential methodological limitation in interpreting this large study is that only a single Dinamap reading was utilized for assessment of baseline BP (as opposed to multiple averaged readings and/or the gold standard sphygmomanometer-based casual BP assessments). In addition, the Keane *et al.*^[39] had a mean age of approximately 44 years—as most participants appeared to have a BP that was well within the normal range, it is possible that the BP assessment may have been affected by a limited range or floor effect.

Research conducted in our laboratory has supported relationships between PTSD and elevated BP. In a recently completed project, several CVD risk factors were assessed among relatively young women with PTSD (mean \pm SD, age = 30 ± 8 years), and compared with

two demographically similar groups with depression and no mental illness^[40]. Analyses revealed that SBP levels in the PTSD group were higher than in the no mental illness ($P < 0.001$) and depression ($P < 0.05$) groups. The DBP levels in the PTSD group were greater than the no mental illness group ($P < 0.05$), but were not significantly different than the depression group. This project utilized three standard sphygmomanometer-determined readings to calculate resting BP. The absolute levels of BP were generally in the normal range.

In another study we analyzed data from the United States National Comorbidity Survey to examine whether PTSD is significantly associated with hypertension, and whether this association is independent of depression^[31]. The study sample ranged in age from 15-54 years and was designed to be representative of the United States population. A total of 4008 respondents were identified who fit into one of four diagnostic groups: (1) history of PTSD diagnosis (lifetime) and no history of major depression ($n = 219$); (2) a lifetime history of both PTSD and major depression ($n = 210$); (3) a history of major depression (lifetime) and no PTSD ($n = 785$); and (4) no history of mental illness ($n = 2794$). The sample was 45% male. In this relatively young sample, the rate of hypertension was modest (7.8% overall). The group with a history of PTSD and no history of depression had the highest rate of hypertension (14.5%), and this rate was significantly higher than the rate in the no mental illness group (6.5%) and the group with history of depression and no PTSD (9.7%). These differences in hypertension rates were significant when controlling for the relationship between age and hypertension rate. The observation that the rate of hypertension between the PTSD no depression group and the PTSD plus depression group (13.9%) was not significantly different, suggested that the relationship of PTSD to high BP is independent of comorbid depression.

STRESS REACTIVITY AND PTSD

Exaggerated cardiovascular reactivity (CVR) in response to psychological stress is associated with markers for CVD such as hypertension, endothelial dysfunction, autonomic nervous system (ANS) dysregulation, and hypothalamic-pituitary-adrenal axis (HPA) alterations^[41-45]. Evidence of physiological reactivity in individuals with PTSD, during trauma reminders, points to CVR as one of the intervening variables between PTSD and the development of CVD^[46,47].

The literature provides evidence for the role of the sympathetic and parasympathetic nervous system dysregulation in PTSD. The roles of PTSD-related hyperarousal and re-experiencing symptoms in producing exaggerated CVR have been a central focus of PTSD/CVD research^[46]. Tucker *et al.*^[48] found greater autonomic reactivity in participants with PTSD than gender-matched trauma exposed controls. In this study^[48], SBP after trauma script delivery was the best measure for classifica-

tion of patients with PTSD (75% sensitivity) and trauma exposed controls (100% specificity).

Chronic autonomic activation leads to dysregulation of the HPA axis in PTSD, which may begin a cascade of physiological responses increasing allostatic load and promoting CVD^[42,49,50]. In response to acute stress, glucocorticoids (GC), primarily cortisol, are involved in both mobilization of defensive resources and in helping the body to return to homeostasis^[50]. Additionally, lowered cortisol levels shortly after traumatic events have been linked to increased risk of developing PTSD following a traumatic event^[51-54]. A recent meta-analysis^[53] of HPA function in PTSD identified significant differences in both basal cortisol and GC receptor sensitivity among individuals with PTSD relative to both trauma-exposed (TC) and non-TC controls (NTC). Specifically, individuals with PTSD showed reduced morning cortisol levels (compared with both TC and NTC), and enhanced GC sensitivity (compared to NTC) as measured by cortisol levels following the dexamethasone suppression test.

Implication of reduced parasympathetic control in individuals with PTSD is evidenced by the negative association between baroreceptor sensitivity and basal HR^[47,55]. Findings of lower HR variability among PTSD groups may provide evidence of autonomic dysregulation due to increased sympathetic hyperactivation and reduced parasympathetic activity^[55-58].

IMMUNE FUNCTIONING IN PTSD

Chronic alterations of neuroendocrine and inflammatory processes have been posited as one mechanism through which risk for CVD is elevated in PTSD. In addition to sympathetic nervous system (SNS) components such as epinephrine and norepinephrine, two interrelated stress-response systems-the HPA axis and the immune system-have been studied in relationship to traumatic stress and posttraumatic outcomes. Both the SNS and HPA axis modulate immune function through several mechanisms, including stimulating proliferation of T-cells and inducing the release of signaling proteins known as Interleukins (IL) or cytokines^[59]. Elevations of pro-inflammatory cytokines, such as IL-6, tumor necrosis factor- α , IL-1 β , and IL-2, as well as downstream acute-phase hepatic proteins such as C-reactive protein (CRP) and fibrinogen, are known to be involved in promoting inflammation, and chronic elevations have been linked to cardiovascular disease risk and other chronic diseases^[42,60,61]. A 2012 review of the literature^[62] indicated that, despite methodological and measurement differences, most studies reported positive associations between pro-inflammatory cytokine concentrations and PTSD symptomatology. Since this review, several studies have provided additional evidence of increased pro-inflammatory cytokines in PTSD^[63-66], although others have reported either no significant relationship^[21,67] or a negative association^[68,69] with PTSD symptoms. The findings related to CRP have been more equivocal, with

recent results ranging from decreased CRP^[70] or no difference^[71] to increased CRP in PTSD as compared with healthy controls^[38,72].

In addition to measuring basal cytokine levels, several recent studies have tested stimulated cytokine levels in PTSD either *in vivo*, through hydrocortisone administration^[18], or through *in vitro* cytokine production by immune cells, whether spontaneous, stimulated using a chemical such as phytohemagglutinin A or lipopolysaccharide, or suppressed using an exogenous GC such as dexamethasone^[20,21,68,73]. Promising new areas of research have also begun to identify genetic and epigenetic changes in DNA methylation^[73] and inflammatory pathways (*e.g.*, nuclear factor- κ B^[73,74]) that may be involved in the risk of PTSD and inflammation-related chronic disease.

Although PTSD seems to be linked to a variety of inflammatory biomarkers, limited preliminary evidence suggests that successful psychological and/or pharmacological treatment of PTSD may result in an abatement of systemic inflammatory responses. Tucker *et al*^[75] first described significant decreases in circulating pro-inflammatory IL-1 β and increases in anti-inflammatory soluble IL-2 receptors after treatment with one of two SSRI medications or placebo. However, another SSRI treatment study did not find any significant post-treatment changes in cerebrospinal fluid levels of IL-6^[76], despite achieving complete remission of PTSD symptoms. A cross-sectional study comparing women in recovery from PTSD to NTC and participants with current PTSD found elevated circulating IL-6 and CRP in current PTSD but identical levels for the recovery and NTC groups^[66]. A longitudinal case-study of one year of psychotherapy also found decreases in excreted IL-6 over time, which seemed to correspond with gradual symptom improvements^[77]. Additionally, following a four-week stress management intervention for survivors of childhood sexual abuse, Wilson^[78] found a modest but statistically significant increase in salivary secretory Immunoglobulin A, a secreted biomarker involved in viral and bacterial immunity^[79].

CONCLUSION

Considering the evidence reviewed in the present article, there appears to be considerable metabolic, autonomic and immune involvement in the elevated CVD risk among individuals with PTSD. There is a high level of agreement among studies that PTSD is positively associated with metabolic syndrome. Stress-related cellular dysfunction may contribute to metabolic syndrome in PTSD^[80]. Dysfunction related to stress-induced dysregulation of telomere/telomerase maintenance, mitochondria, and endoplasmic reticular stress may result in metabolic syndrome^[81-83]. Conceptualizing the CVD risk factors from the standpoint of metabolic syndrome allows one to fully appreciate the clinical significance of multiple interacting physiological risks in PTSD^[26,28]. In short, the impact of multiple risk factors is synergistic, resulting in a magnitude of risk greater than the sum of the individual risk factors.

Although findings concerning BP in PTSD are mixed, the overall direction of this relationship appears to be positive, with greater rates of hypertension in PTSD. Methodological factors in the study of resting BP in PTSD may have masked the extent of this problem. Additional studies across the range of BP levels (*i.e.*, normal, elevated, and high) may provide more insight into the extent of BP differences and prevalence of elevated BP in PTSD, as well as the mechanisms by which BP elevation occurs in early age.

The available evidence also suggests a positive relationship between PTSD and autonomic reactivity. Although further research is needed to fully elucidate the role of ANS stress reactivity in PTSD, recent advances suggest that sympathetic and parasympathetic dysfunction in PTSD may be evident through some reactivity paradigms^[56,57]. The burgeoning literature on immune functioning in PTSD is rapidly providing insights into additional mechanisms (*e.g.*, proinflammatory cytokines and other immune biomarkers) that assist in understanding the relationships of PTSD to illnesses such as CVD^[21,62,66]. In all, the available studies indicate a significant relationship between PTSD and immune dysfunction. With regard to future directions in the area of PTSD and CVD risks, further research on the role of ANS reactivity in PTSD-related CVD risk, as well as approaches to prevention and management of CVD risk factors in this population, would represent advanced directions in the field.

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Antioxidants, inflammation and cardiovascular disease

Harald Mangge, Kathrin Becker, Dietmar Fuchs, Johanna M Gostner

Harald Mangge, Research Unit on Lifestyle and Inflammation associated Risk Biomarkers, Clinical Institute of Medical and Chemical Laboratory Diagnosis, Medical University of Graz, 8036 Graz, and BioTechMed-Graz, Austria

Kathrin Becker, Dietmar Fuchs, Division of Biological Chemistry, Biocenter, Innsbruck Medical University, 6020 Innsbruck, Austria

Johanna M Gostner, Division of Medical Biochemistry, Biocenter, Innsbruck Medical University, 6020 Innsbruck, Austria

Author contributions: Each of the authors has made a substantial contribution so as to qualify for authorship; and all authors have read and approved the paper.

Correspondence to: Dr. Dietmar Fuchs, Division of Biological Chemistry, Biocenter, Innsbruck Medical University, Innrain 80, 6020 Innsbruck, Austria. dietmar.fuchs@i-med.ac.at

Telephone: +43-512-900370350 Fax: +43-512-900373110

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Abstract

Multiple factors are involved in the etiology of cardiovascular disease (CVD). Pathological changes occur in a variety of cell types long before symptoms become apparent and diagnosis is made. Dysregulation of physiological functions are associated with the activation of immune cells, leading to local and finally systemic inflammation that is characterized by production of high levels of reactive oxygen species (ROS). Patients suffering from inflammatory diseases often present with diminished levels of antioxidants either due to insufficient dietary intake or, and even more likely, due to increased demand in situations of overwhelming ROS production by activated immune effector cells like macrophages. Antioxidants are suggested to beneficially interfere with diseases-related oxidative stress, however the interplay of endogenous and exogenous antioxidants with the overall redox system is complex. Moreover, molecular mechanisms underlying oxidative stress in CVD are not fully elucidated. Metabolic dybalances are suggested to play a major role in disease onset and progression. Several central signaling

pathways involved in the regulation of immunological, metabolic and endothelial function are regulated in a redox-sensitive manner. During cellular immune response, interferon γ -dependent pathways are activated such as tryptophan breakdown by the enzyme indoleamine 2,3-dioxygenase (IDO) in monocyte-derived macrophages, fibroblasts, endothelial and epithelial cells. Neopterin, a marker of oxidative stress and immune activation is produced by GTP-cyclohydrolase I in macrophages and dendritic cells. Nitric oxide synthase (NOS) is induced in several cell types to generate nitric oxide (NO). NO, despite its low reactivity, is a potent antioxidant involved in the regulation of the vasomotor tone and of immunomodulatory signaling pathways. NO inhibits the expression and function of IDO. Function of NOS requires the cofactor tetrahydrobiopterin (BH_4), which is produced in humans primarily by fibroblasts and endothelial cells. Highly toxic peroxynitrite ($ONOO^-$) is formed solely in the presence of superoxide anion (O_2^-). Neopterin and kynurenine to tryptophan ratio (Kyn/Trp), as an estimate of IDO enzyme activity, are robust markers of immune activation *in vitro* and *in vivo*. Both these diagnostic parameters are able to predict cardiovascular and overall mortality in patients at risk. Likewise, a significant association exists between increase of neopterin concentrations and Kyn/Trp ratio values and the lowering of plasma levels of vitamin-C, -E and -B. Vitamin-B deficiency is usually accompanied by increased plasma homocysteine. Additional determination of NO metabolites, BH_4 and plasma antioxidants in patients with CVD and related clinical settings can be helpful to improve the understanding of redox-regulation in health and disease and might provide a rationale for potential antioxidant therapies in CVD.

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Key words: Atherogenesis; Cardiovascular disease; Neopterin; Nitric oxide; Tetrahydrobiopterin; Tryptophan; Oxidative stress; Homocysteine; Vitamins; Antioxidative therapy

Core tip: Crosstalk between a number of pathways in

volved in the regulation of immune and endothelial homeostasis is strongly coordinated by redox processes. Underlying molecular mechanisms of atherogenesis include metabolic imbalances that are linked to the onset and progression of endothelial dysfunction and inflammation, finally leading to a status of heightened oxidative stress. Decrease of plasma antioxidants may develop secondarily due to an increased demand for oxidation-sensitive vitamins during inflammation. Antioxidant and vitamin supplementation therapy is controversially discussed and success might depend of an individual patient's demand.

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INTRODUCTION

Despite the availability of successful treatment strategies for dyslipidemia and hypertension, cardiovascular diseases (CVD) account for one third of all deaths worldwide, and prevalence still increases^[1,2].

CVD comprise a class of diseases that involve heart and systemic blood vessels^[3]. In coronary heart disease, cerebrovascular disease or peripheral arterial disease, impaired blood vessel function leads to an inadequate blood supply of organs. Deep vein thrombosis and pulmonary embolism are usually caused by blood clots in the leg veins.

Avoiding risk factors such as smoking, obesogenic lifestyle, *e.g.*, unhealthy diet, physical inactivity, high blood pressure, diabetes and dyslipidemia, is strongly recommended for disease prevention. Nevertheless, beside lifestyle, genetic, epigenetic and environmental factors may essentially influence the risk of CVD.

The multifactorial background makes it difficult to unravel initial pathological events, which are suggested to occur in a very early phase of disease, where symptoms are subclinical. Inflammation is considered to play a key role in both disease initiation and progression^[4]. Chronic inflammatory conditions attenuate endogenous antioxidant capacities due to continuous production of high levels of reactive oxygen species (ROS). Patients often represent with low blood levels of antioxidants^[5] and enhanced oxidative stress markers^[6]. This is usually due to increased demand in situations of overwhelming ROS production by activated immune effector cells like macrophages. Also insufficient nutritional intake may play a role. Uptake of exogenous antioxidants is suggested to beneficially interfere with diseases-related oxidative stress, however the interplay of endogenous and exogenous antioxidants with the overall redox system is complex.

The object of this review is to give an overview on immunobiochemical pathways activated in atherogen-

esis, which lead to oxidative stress-related pathological consequences. Understanding of the mechanisms will be helpful in the establishment of new preventive and therapeutic strategies.

MAIN FEATURES OF ATHEROGENESIS

Atherosclerosis is the most common pathological process that leads to CVD including myocardial infarction (MI), heart failure, stroke and claudication. A central event is the development of atherosclerotic plaques in the inner lining of arteries. Irritative inflammatory stimuli, hypertension, hyperglycemia and dyslipidaemia cause endothelial stress leading to expression of adhesion molecules and recruitment of leukocytes^[7].

Atherosclerotic plaques are characterized by necrotic cores, calcification, accumulation of modified lipids and foam cells, but also other cell types such as smooth muscle cells, vascular dendritic cells, T cells and endothelial cells are involved in lesion formation^[8]. The "oxidative modification hypothesis" of atherogenesis implies that low-density lipoprotein (LDL) oxidation is an early event in atherosclerosis^[9]. Cholesterol-containing LDL particles are retained in the artery wall and biochemically modified components of these particles in turn induce leukocyte adhesion but also intracellular cholesterol accumulation in invaded macrophages^[10]. Chronic inflammatory conditions are maintained due to the production of pro-inflammatory mediators through immune competent cells in the lesions^[11]. Activation of macrophages is a key factor in atherosclerotic plaque formation, fibrous cap disruption and thrombus formation.

While in the past atherosclerosis was viewed primarily as passive process of cholesterol accumulation, recent evidence indicates that it is a highly active process involving components of the vascular, immune, metabolic and endocrine system^[12]. Initial pathological changes occur in a variety of cell types long before symptoms become apparent and diagnosis is made^[13,14]. Of note, also in a large sample of cardiovascular disease-free adults, Chrysoschoou *et al*^[15] revealed an association of pre-hypertension with reduced serum antioxidant capacity and increased oxidized LDL probably indicating initial pathological changes.

Atherosclerotic plaque composition, endothelial erosion, intraplaque hemorrhage, adventitial and intraplaque neovascularization, rather than the percentage of stenosis, turned out to be critical predictors for both risk of plaque rupture and subsequent thrombogenicity^[12,16,17]. Disruption of a vulnerable or unstable plaque may lead to a complete occlusion, to plaque progression or result in an acute coronary syndrome, *i.e.*, acute MI (AMI), unstable angina and sudden cardiac death or stroke in case of carotid plaque destabilization.

OXIDATIVE STRESS AND IMMUNE ACTIVATION

Although substantial efforts have been made to dissect

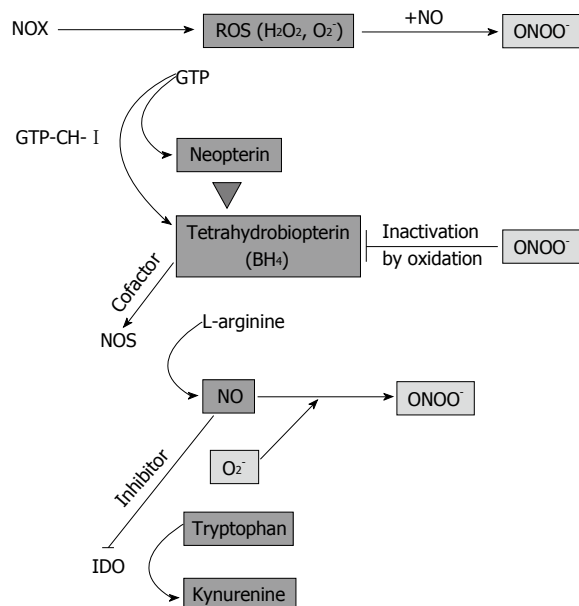


Figure 1 Regulatory circuits in inflammation and endothelial dysfunction. During inflammation, NADPH oxidase (NOX) produces high levels of reactive oxygen species (ROS). T cells and natural killer cells produce interferon- γ , which activates enzyme GTP-cyclohydrolase I (GTP-CH- I), indoleamine 2,3-dioxygenase (IDO) and inducible nitric oxide synthase (iNOS) in monocyte-derived macrophages (M) and dendritic cells (DC). In endothelial cells, endothelial NOS (eNOS) is constitutively expressed and GTP-CH- I produces tetrahydrobiopterin (BH₄), which is a NOS cofactor. BH₄ deficiency leads to NOS uncoupling and superoxide anion (O₂⁻) formation, which reacts with NO to form peroxynitrite (ONOO⁻). In a vicious cycle, ONOO⁻ oxidizes BH₄. In M/DC, GTP-CH- I synthesizes neopterin at expense of BH₄, which contributes to the low activity of iNOS in human M/DC. Furthermore, NO is a reversible inhibitor of the immunoregulatory enzyme IDO. IDO degrades the essential amino acid tryptophan to kynurenine.

molecular details of atherogenesis, a full understanding of the underlying mechanisms is still missing. However, activation of immune competent cells, leading to local and finally systemic inflammatory phenomena and the associated status of heightened oxidative stress are central events^[4].

Monocyte/macrophage accumulation at the lesion is a key factor in the pathology and involves several steps, such as monocyte recruitment by expression of adhesion molecules and chemotactic factors, induction of activation and differentiation processes as well as proliferation, and immobilization of macrophages in the inflamed plaque^[18]. Current data indicate that due to the presence of variable differentiation stimuli, different macrophage populations reside in the atherosclerotic plaque^[19]. Both M1 and M2 macrophages are present in atherosclerotic regions. Macrophage colony-stimulating factor (M-CSF), which is continuously present in circulation, induces predominantly M2-type macrophages with increased phagocytic activity, characterized by expression of interleukin (IL)-10, scavenger receptor A and mannose receptor^[18,19]. Granulocyte-macrophage CSF (GM-CSF) induces M1-polarized cells with antigen presentation capacities, which express tumor necrosis factor alpha (TNF α) and pro-inflammatory cytokines such as IL-1 β , IL-6, IL-8 and

IL-12 upon stimulation with interferon gamma (IFN- γ) or lipopolysaccharides (LPS)^[18,20]. While M1 macrophages were found to predominate in rupture-prone plaque regions, M2-type are located in the vascular adventitial tissue^[21]. However, also other macrophage phenotypes are suggested to contribute to the inflammatory process in atherosclerosis, such as the recently described platelet chemokine CXCL4-induced M4 cells^[22].

Immune reactions in atherosclerotic lesions are mainly T helper (Th1) type responses, as indicated by the dominance of pro-inflammatory and macrophage-stimulating cytokines found in advanced plaques^[11,23,24]. During Th1-type response, IFN- γ is probably the most important trigger for high ROS production in macrophages^[25] by phagocytic NADPH oxidase (NOX)^[26]. Main reactive species are hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻), but also reactive nitrogen species such as peroxynitrite (ONOO⁻), nitrogen dioxide and trioxide^[27]. IFN- γ signaling initiates a variety of cellular defense mechanisms such as pro-inflammatory cytokine production *via* nuclear factor kappa B (NF- κ B) signaling, enhancement of antigen presentation^[28] and other important pathways, *e.g.*, neopterin formation *via* guanosine triphosphate (GTP)-cyclohydrolase I (GTP-CH- I) and indoleamine 2,3-dioxygenase (IDO)-mediated tryptophan breakdown^[29] (Figure 1).

Under normal conditions, low levels of ROS are mainly byproducts from electron transport chain reactions in the mitochondria^[30]. They are important regulators of several redox-sensitive pathways involved in the maintenance of cellular homeostasis^[31], and act by modifying molecules, enzymes and transcription factors as well by interfering with the endogenous antioxidant pool^[27,31,32]. Depletion of endogenous redox buffer systems in conditions with overwhelming oxidative stress is critical, not only due to triggering of immune responses but also through leading to endothelial and smooth muscle dysfunction, and thus to the progression of atherosclerosis^[33,34].

ROLE OF LIPOPROTEINS IN ATHEROSCLEROSIS

Proatherogenic oxidized LDL (oxLDL) accumulates in the vascular wall and contributes to the pathogenesis of vascular dysfunction early in the development of atherosclerosis. After incorporation *via* scavenger receptors of macrophages, oxLDL leads to their transformation into foam cells^[35]. Foam cells accumulate a variety of lipids in droplets in the cytoplasm and secrete extracellular matrix proteins that further support the retention of lipoproteins and attraction of immune cells, thus leading to an enlargement of the lesion^[10].

Oxidation of LDL is considered to occur primarily in the vascular wall^[36], but also circulating oxLDL was detected in CVD patients^[37]. Although the amount of circulating oxLDL is small compared to oxLDL present in vessels^[38], enhanced serum levels of oxLDL are predictive for endothelial dysfunction and coronary heart dis-

ease^[36-39]. The role of oxLDL as a relevant pro-atherogenic marker is further supported by the study of Meisinger *et al.*^[40], who found elevated oxLDL to be predictive for future coronary heart disease events in apparently healthy men. Oxidation of LDL contributes to the prooxidant environment in atherosclerotic lesions. OxLDL is a potent stimulus of vascular ROS formation, mainly through activation of NOX and uncoupling of endothelial nitric oxide (NO)-synthase (NOS) that promotes endothelial dysfunction^[36].

High-density lipoprotein (HDL) is another potential biomarker with anti-atherogenic properties due to its function in the reverse cholesterol transport and in decreasing lipoprotein oxidation^[41]. HDL is involved in several biological processes that counteract inflammation and oxidative stress, by beneficially influencing, *e.g.*, pancreatic beta-cell function, endothelial vasoreactivity, endothelial apoptosis, restorative processes and monocyte activation as well as adhesion molecules expression, thus being highly vasculoprotective^[42]. Paraonase-1, a calcium dependent enzyme, is located at the surface of HDL particles and contributes to the antioxidant and anti-inflammatory role of HDL^[43]. In particular, HDL-associated paraonase was shown to inhibit the formation of “minimally oxidized” LDL^[44]. Nevertheless, also other mechanisms are suggested to be involved in HDL-associated inhibition of LDL oxidation^[45].

Plasma HDL cholesterol (HDL-C) levels are inversely associated with CVD risk in preclinical and large epidemiologic studies. Low HDL-C level was identified as a robust predictor of lipid peroxidation irrespective of gender, age, obesity and inflammatory or metabolic biomarkers in the Styrian Juvenile Obesity/ Early DEtec-Tion of Atherosclerosis study employing 797 participants aged from 5 to 50 years^[46]. However, HDL is highly heterogeneous and the atheroprotective functions of the different HDL subpopulations are not completely understood. Furthermore, current data indicate that therapeutically increased HDL-C levels *per se* do not always correlate with enhanced HDL functions *in vivo*^[47,48].

Of note, accumulation of free, unesterified cholesterol can lead to crystal formation both *in vitro* and *in vivo*^[49]. Crystallized cholesterol in atherosclerotic plaques was shown to activate the NLR family, pyrin domain containing 3 (NLRP3) inflammasome complex by employing the complement system, thereby leading to the release of proinflammatory cytokine IL-1 β ^[50,51]. Cholesterol crystals were mainly found in advanced plaques, however the inflammatory responses caused by NLRP3 inflammasome activation might represent an important trigger in disease progression and could thus represent an important pharmaceutical target^[52].

NEOPTERIN FORMATION

Neopterin, a marker of immune system activation, is produced by GTP-CH- I in macrophages and dendritic cells (DC)^[53,54] and has emerged as an important independent and predictive marker in cardiovascular risk assessment^[6].

IFN- γ is the major stimulus for neopterin formation. Other cytokines have only limited stimulatory potential *in vitro* but some, *e.g.*, TNF α , can indirectly enhance IFN- γ induced neopterin formation^[55]. Of note, also pro-inflammatory compounds like LPS can elevate neopterin levels^[55]. The amount of neopterin secreted by human macrophages correlates with their ROS-generation capacity *in vitro*^[56] and neopterin concentration in body fluids is considered as an indicator for immune activation-associated oxidative stress^[57].

GTP-CH-1 catalyzes the conversion of guanosine triphosphate (GTP) to 7,8-dihydroneopterin triphosphate finally leading to the formation of neopterin, 7,8-dihydroneopterin and 5,6,7,8-tetrahydrobiopterin (BH₄)^[57]. Human monocyte-derived macrophages and DCs are the most important source of neopterin and its partially reduced derivative 7,8-dihydroneopterin, both present in relative constant ratio in human serum^[57], but not of BH₄, due to the relative deficiency of pyruvoyl-tetrahydropyrimidine synthase in this cell types^[58] (Figure 1). In contrast, cells from other animal species and other human cell types such as endothelial cells or fibroblasts preferentially produce BH₄, which is needed as a cofactor by several monooxygenases including NOS, phenylalanine hydroxylase or tyrosine hydroxylase^[59].

Elevated neopterin concentrations were reported to be associated with chronic immune activation in several diseases such as viral, bacterial and parasite infections, autoimmune or malignant tumor diseases and during rejection episodes in allograft recipients^[60-63]. Also patients with CVD present with increased neopterin concentrations, supporting the crucial involvement of chronic immune activation, in particular of macrophages, in atherogenesis. Several studies (Table 1) strengthened the impact of neopterin as an independent marker for CVD, with predictive value for coronary artery disease (CAD) progression^[6].

Of note, neopterin-positive macrophages were found in coronary atherectomy specimens from patients with stable angina pectoris and to a lesser extent in those with unstable angina pectoris, and the number of these macrophages correlated with T cell and neutrophil count in the lesions^[76]. Furthermore, neopterin was shown to induce an atherothrombotic phenotype in human coronary endothelial cells *in vitro* by promoting cellular adhesion molecules (intercellular adhesion molecule 1 and vascular cell adhesion molecule 1) and tissue factor (TF) expression mediated by activation of NF- κ B^[77]. These data suggest that neopterin is not only associated with the systemic inflammation process in atherosclerosis, but might also be of importance for the inflammatory process within the plaque and thus for plaque destabilisation^[6,76].

Neopterin concentrations correlate with IFN- γ -induced ROS production^[56]. In addition, neopterin has pro-oxidant properties itself by intensifying the effects of ROS as well as of reactive chlorine and nitrogen species^[78]. Of note, Herpfer *et al.*^[79] showed that neopterin is able to enhance ONOO⁻ as well as Cu(II)-mediated LDL oxidation, whereas 7,8-dihydroneopterin may protect

Table 1 Selected studies investigating neopterin concentrations in cardiovascular disease patients

Ref.	Condition	n	Result
Melichar <i>et al</i> ^[64] , 1994	AMI	13	Increased urinary neopterin
Anwaar <i>et al</i> ^[65] , 1999	Acute cerebral ischemia or transient ischemic attack, 1-yr follow-up	59 (57)	Increase of plasma neopterin after acute cerebral ischemia
Tatzber <i>et al</i> ^[66] , 1991	Different clinical stages of atherosclerosis	61	Elevated plasma neopterin in about 50% of hospitalized patients undergoing conservative or surgical therapy, higher neopterin levels were overrepresented in patients with higher Frederickson type
Weiss <i>et al</i> ^[67] , 1994	Cross-sectional community-based screening study (Ischemic Heart Disease and Stroke Prevention Study, Bruneck, Italy)	561 (total)	Serum neopterin correlated with the extent of carotid atherosclerosis
Schumacher <i>et al</i> ^[68] , 1997	AMI	21	Neopterin levels were highest in AMI patients but also elevated in those with CAD
Gurfinkel <i>et al</i> ^[69] , 1999	Stable CAD	62	
	Unstable angina pectoris (non-Q-wave AMI)	52 (26)	Serum neopterin correlated with score of atherosclerotic extension (angiography)
Zouridakis <i>et al</i> ^[70] , 2004	Chronic stable angina pectoris	124	CAD progression correlated with increased neopterin and high-sensitivity C-reactive protein as well endothelial activation markers
Avanzas <i>et al</i> ^[71] , 2005	Patients with chronic stable chest pain undergoing diagnostic coronary angiography, 1-yr follow-up	297	Elevated serum neopterin correlated with adverse coronary events during follow-up (17.2%)
Kaski <i>et al</i> ^[72] , 2008	NSTE ACS (unstable angina and NSTE MI), 6-mo follow-up	397 (147,250)	Baseline neopterin in unstable angina and NSTE MI comparable, increased neopterin was associated with adverse cardiac events
Johnston <i>et al</i> ^[73] , 2006	ACS (treatments: medication, uncoated or rapamycin-eluting coronary stents) and stable CAD	70 (35, 25, 10)	Serum neopterin correlated with thrombolysis in myocardial infarction; mean changes in serum neopterin higher in uncoated stent group
Barani <i>et al</i> ^[74] , 2006	Critical limb ischemia, 1-yr follow-up	36 (232)	Neopterin was elevated in patients with atrial fibrillation or flutter and with ischemic electrocardiogram changes which were at risk for adverse cardiac events
Ray <i>et al</i> ^[75] , 2007	ACS (PROVE IT-TIMI 22) 2-yr follow-up	3946	Increased neopterin was associated with increased risk of death and of acute coronary events after ACS

NSTE: Non-ST-segment elevation; PROVE IT-TIMI: PRavastatin or atorVastatin Evaluation Infection Therapy-Thrombolysis In Myocardial Infarction; AMI: Acute myocardial infarction; MI: Myocardial infarction; CAD: Coronary artery disease; ACS: Acute coronary syndrome. Table adapted and extended from Fuchs *et al*^[6].

LDL from oxidation under certain conditions^[79,80]. Neopterin may also enhance the effects of ONOO⁻ in the processes of protein nitration^[81]. This pro-oxidant property of neopterin indicates a potential involvement in the antimicrobial and antitumoral action of macrophages^[82]. The property of neopterin to interfere with and enhance the effects of various ROS might be of central relevance also in atherogenesis.

Inflammation-associated oxidative stress may lead to a rapid consumption of circulating antioxidants. In patients with CAD, higher neopterin concentrations were associated with a decline in levels of several antioxidant compounds and vitamins such as ascorbic acid, α -tocopherol, lycopene, lutein and zeaxanthin^[5].

TRYPTOPHAN BREAKDOWN

In parallel to neopterin formation, other IFN- γ -dependent pathways are activated during cellular immune response such as tryptophan breakdown by IDO. IDO catalyzes the rate-limiting step in the conversion of tryptophan (Trp) and other indole derivatives to kynurenine (Kyn)^[83] and is induced in monocyte-derived macrophages but also in fibroblast, endothelial and epithelial cells^[84,85] (Figure

1). Both expression and activity of the haeme-containing enzyme IDO is sensible to redox-regulation and IDO enzyme itself can exert antioxidant activity by scavenging of O₂⁻^[86,87]. The estimation of Kyn to Trp ratio (Kyn/Trp), expressed as μ mol kynurenine per mmol tryptophan, can be used as measure of IDO enzyme activity both *in vitro* and *in vivo*^[60,88]. Simultaneous measurement of immune activation markers such as neopterin, IFN- γ or soluble interleukin receptors, allow to relate circulating Trp levels with inflammation-induced IDO activity, as also hepatic tryptophan 2,3-dioxygenase (IDO) could degrade Trp. IDO, however, is regulated *via* tryptophan content and steroid hormones such as glucocorticoids^[89,90], while IDO is strongly induced in response to several pro-inflammatory stimuli such as IFN- γ , TNF α or LPS^[55,85].

Depletion of the essential amino acid Trp contributes to the development of an antiproliferative environment and represents an effective antimicrobial and antitumoral strategy^[91]. Also T cell proliferation depends on Trp availability, thus IDO activation is a metabolic checkpoint of immunoregulation^[92]. IDO activity is crucially involved in the control of T cell proliferation and in the generation of regulatory T cells, and thus in the suppression of autoimmune responses and promotion of tolerance^[92,93].

Metabolic control by reduction of Trp levels may slow down hematopoiesis in addition to other proinflammatory stimuli by affecting the growth and differentiation of erythroid progenitor cells. In line with this, in patients with inflammation-induced anemia, Kyn/Trp was found to inversely correlate with haemoglobin levels^[94,95].

Accelerated Trp breakdown was reported in patients with coronary heart disease^[96] and IDO activity correlated significantly with several risk factors for atherosclerosis in the Cardiovascular Risk in Young Finns Study^[97]. Niinsalo *et al.*^[98] reported that IDO activity positively correlated with carotid artery intima/media thickness, an early marker of atherosclerosis, although this association did not remain significant after adjustment with classical risk factors in this patient group.

In inflammatory diseases including CVD, a concurrent increase of neopterin production and tryptophan degradation is usually observed. The prognostic ability of neopterin is likely to relate to the association with IFN- γ in the atherogenic process^[6]. IDO-mediated tryptophan breakdown is suggested to be responsible for several additional aspects observed during disease progression^[29], *e.g.*, the development of depression. Because tryptophan is a precursor for the biosynthesis of serotonin, the lowered tryptophan availability under inflammatory conditions may limit serotonin formation and thus enhance the susceptibility for lowered mood and depression^[99]. Of note, development of depressive symptoms have been associated with increased CAD risk and poor prognosis^[100]. Estimation of Trp breakdown rate could contribute to a better understanding of the interplay between inflammation, metabolic syndrome, mood disturbance, and anemia, all previously described as significant predictors of an unfavorable outcome in patients with CVD^[101].

NITRIC OXIDE

NOS converts L-arginine into citrulline, thereby synthesizing NO. Free NO can migrate through cell membranes by diffusion, and although it relatively low reactivity, NO is a potent antioxidant molecule that can protect from ROS damage^[102]. However, NO is a free radical, and can undergo oxidation to nitrite and nitrate, react with O₂⁻ to form ONOO⁻, or bind to transition metals^[103]. NO signaling is strongly concentration dependent and although endogenous NO is essentially involved in many physiological processes and beneficial in a variety of circumstances, its reaction products may mediate nitrosative and oxidative stress. However, NO products can have also protective effects. In plasma, NO circulates primarily complexed in S-nitrosothiol species^[104] that are suggested to be a transport and buffer system that controls intercellular NO exchange. S-nitrosylation of the proteome is a unique form of posttranslational modification that can have significant consequences for protein function and cell phenotype. In particular in the cardiovascular system, S-nitrosothiols were shown to exert many actions, including promoting

vasodilation, inhibiting platelet aggregation, and regulating Ca(2+) channel function of myocytes^[105]. The impact of S-nitroso but also N-nitroso protein formation on the reduction of free NO under inflammatory conditions *in vivo* has still to be investigated^[106,107].

Endothelial and neuronal NOS are constitutively expressed and produce NO at low concentrations, while inducible NOS is activated, *e.g.*, in macrophages of several species in response to pro-inflammatory stimuli giving rise to higher NO output^[108]. Endothelial dysfunction, *e.g.*, vasodilation and/or platelet inhibition, a key feature of early atherosclerosis, is associated with the reduced availability of endothelium-derived NO^[109]. Defects in NO production, metabolism and response have been described to be responsible mechanisms.

In the presence of O₂⁻, ONOO⁻ formation may be a factor that limits NO bioavailability. Beside being strongly vasoconstrictory, ONOO⁻ has been shown to oxidize the NOS cofactor BH₄, thereby leading to eNOS uncoupling and O₂⁻ production^[110], thus starting a vicious cycle (Figure 1). Reduced vascular BH₄ levels were found in rat and mice models of atherosclerosis and diabetes^[111].

High NO output and generation of reactive nitrogen species *via* iNOS contribute to cytotoxic defense strategies in inflammation. However, although this has been reported for several species, including mice, until now, large output of NO by iNOS could not be equally demonstrated in human macrophages^[112,113]. Human macrophages produce neopterin at the expense of BH₄, and low BH₄ leads to NOS enzyme uncoupling. Furthermore, the pro-oxidant properties of neopterin may compensate for deficient NO and ONOO⁻ production^[114].

Of note, NO inhibits IDO expression and function by reversibly binding to the active site heme^[115]. Induction of IDO and NOS in IFN- γ -mediated inflammatory response is suggested to be functionally cross-regulated^[116]. The absence of NO-mediated immunoregulation may support enhanced IDO activity at the site of inflammation.

POTENTIAL ROLE OF TH2 RESPONSES IN CVD

Th1 responses are in general proinflammatory and known to be proatherogenic, while Th2 cells are usually involved in helminth and allergic responses. The role of Th2 cells in atherosclerosis seems to be very complex and even contradictory. A potential protective role of Th2 response is discussed in few studies^[117,118], while Ait-Oufella *et al.*^[24] assume a potential proatherogenic function of Th2 cells within the complex interaction theater of CD4⁺ T cell subsets in atherosclerosis. Thus, the exact role of the Th2 response remains to be elucidated based on an improved understanding of the complex interplay between Th1, Th2, and other T cell populations such as Th17 and Tregs within the atherosclerotic scenario^[18,24]. Overall, Th cell subset polarization in atherosclerosis is less distinct in humans compared to mice^[119].

High cholesterol diet of ApoE(-/-) mice with differ-

ent T cell subset polarization resulted in increased development of atherosclerosis in the aortic root and abdominal aorta in mice with predominantly Th1-like immune responses [ApoE(-/-) BL/6 mice] in comparison to animals with Th2 predominance [ApoE(-/-) BALB/c]^[120]. A potential of IL-4 to limit Th1 cell responses and reducing lesion size was observed in several murine atherosclerotic models^[121,122].

Only recently, Engelbertsen *et al*^[123] reported an association between Th2 immunity and reduced risk of MI, as high numbers of Th2 cells were associated with decreased mean common carotid intima-media thickness, reduced risk of AMI in women and IL-4 was independently associated with reduced risk of CVD. Although some limitations, as, *e.g.*, differences in lymphocyte number between healthy man and women or the use of long-term cryo-conserved cells, this study provides first hints for the clinical importance of an improved understanding of Th2-type responses in CVD. However, again in contrast to these positive, protective attributes, Shimizu *et al*^[124] suggested a role for Th2 cells and cytokines in the promotion of arterial aneurysm formation.

ANTIOXIDANTS IN CVD THERAPY

Oxidative stress triggers inflammation and endothelial disruption in atherogenesis. A number of studies showed that exogenous antioxidants can modulate endothelium-dependent vasodilation responses, endothelium-leukocyte interactions as well as balance between pro- and anti-thrombotic properties^[125]. Accordingly, antioxidant therapy was suggested to beneficially interfere with development and progression of atherosclerosis.

Th1/Th2 balance is crucially dependent on redox-events; while Th1 responses prevail at oxidative conditions, Th2 responses were shown to be supported by “antioxidative stress”^[126]. Thus, disequilibrium of Th1/Th2 cytokines may be involved in CVD as a mechanism of immunotoxicity. As Th1 and Th2 reactions crossregulate each other to balance immune responses^[127], suppression of Th1-type response by antioxidants would favour Th2-type reactions. Of note, several types of antioxidant were shown to reduce IFN- γ -stimulated tryptophan degradation and neopterin in peripheral blood mononuclear cells *in vitro*^[87,128].

A number of studies reported an inverse relationship between plasma antioxidants, or total antioxidant capacity and cardiovascular diseases^[5,15]. Low intake of antioxidants, in particular of vitamins, was suggested to be associated with an increased risk of CVD^[129,130]. Thus, the finding of an inverse correlation between concentrations of antioxidant compounds and vitamins and disease risk could relate to an increased requirement for antioxidant molecules during inflammatory diseases and insufficient supply with these compounds may further accelerate disease process. However, this assumption has not been conclusively proven in clinical trials and is still controversially discussed in the literature^[131-134]. Likewise,

equivocal effects of antioxidant supplementation with vitamin E, beta-carotene, alpha lipoic acid, coenzyme Q10, alone or in combination, on cardiovascular health were reported^[135].

Major effects were expected from vitamin E therapy. Due to its fat-solubility, vitamin E is part of cell membranes and lipoprotein particles, where it counteracts oxidation events. Vitamin E-mediated protection from oxidative stress and atherosclerotic plaque formation has been shown both *in vitro* and in mouse models. However, while in several clinical trials vitamin E supplementation lead to a reduction of risk of fatal and nonfatal AMI, others reported even a slight increase of mortality upon high dose vitamin E treatment^[136]. Thus, no final suggestion can be made about the impact of vitamin E supplementation and even recent metanalysis including a large trial number lead to inconsistent results^[137].

So far, although a general association of low vitamin levels and oxidative stress related conditions is established, no clear evidence for a beneficial effect of vitamin supplementation exists. The association between vitamin deficiency in patients and disease symptoms is suggested to result mainly from the inflammation-associated consumption of oxidation-sensitive vitamins^[29,132,138], which may lead to a variety of secondary effects.

Apart from being part of the antioxidant defense system, some vitamins act as enzyme cofactors. Low B vitamin availability (B6, B12 and folic acid) leads to impaired remethylation of homocysteine to methionine and thus to homocysteine accumulation, as they are essential cofactors in homocysteine-methionine metabolism. Increased homocysteine levels were found to be associated with arteriosclerotic outcomes and risk of stroke in elderly individuals^[139], and are considered as an independent risk marker for CVD^[140]. However, lowering homocysteine levels by B-vitamin supplementation failed to demonstrate beneficial effects in CVD^[141]. Also, in open-label study with demented patients on B vitamins, a decline of homocysteine has been observed, while neopterin levels were not affected^[142]. Recent data indicate that homocysteine accumulates secondarily due to heightened oxidative stress associated with immune activation^[143-145]. Thus, also the impact of the selected marker has to be critically evaluated when assessing the effect of vitamin supplementation.

A broader understanding of antioxidant action is clearly warranted. Beside their direct effects in the prevention of biomolecule oxidation by being oxidized themselves, several antioxidants mediate a variety of effects that are of longer duration, as they may induce signaling changes in the biological system^[146]. However, a variety of drugs may act also as antioxidants, thus antioxidant vitamins could interfere with pharmaco-relevant signaling pathways. This is of particular relevance for multi-target drugs such commonly used statins.

A major aim in the treatment of atherosclerosis is the prevention of LDL oxidation. Lipid-lowering compounds such as statins and niacin (vitamin B3, nicotinic

acid) are in use for a long time, alone or together, for cardiovascular protection in patients with coronary disease and low plasma levels of HDL^[147]. However, combination therapies with other antioxidant vitamins seemed even to counteract the beneficial effect of statin/niacin therapy^[147,148].

Statins are inhibitors of 3-hydroxy-3-methylglutaryl-co-enzyme A (HMG-CoA) reductase, and their lipid-lowering effects are suggested to reduce the risk of coronary heart disease^[149], although therapeutic efficacy is controversially discussed^[150].

The primary mechanism of statin action is suggested to be the reduction of LDL cholesterol, but several clinical trials indicate that statins exert pleiotropic effect that contribute to therapeutic efficacy. Statins act as effective antioxidants by inhibiting generation of ROS, but also by interfering with NOX and NOS, antioxidant enzymes, lipid peroxidation and LDL cholesterol oxidation^[151]. In *in vitro* studies with vascular smooth muscle and mononuclear cells, treatment with atorvastatin could reduce NF- κ B activation and expression of pro-inflammatory cytokines and chemokines^[152]. In human peripheral blood mononuclear cells and in monocytic cell lines, atorvastatin was shown to suppress stimulation-induced neopterin formation and tryptophan degradation, suggesting that both immunoreactivity of T cells and of monocyte-derived macrophages are down-regulated by this statin^[153]. Treatment with several statins could promote Th2 polarization of CD4+ T cells primed *in vitro* with anti-CD3 antibody and splenic antigen-presenting cells^[154]. These findings strongly suggest that statins contribute to the regulation of Th1/Th2 cell balance also *in vivo*. In endothelial cells, statins were shown to be involved in restorative processes by increasing NO-bio-availability and promoting re-endothelialization^[155]. Of note, lovastatin was able to prevent neopterin-induced activation of human coronary artery endothelial cells *in vitro* by interfering with NF- κ B activation and decreasing expression of cellular adhesion molecules and TF β ^[177]. Furthermore, lovastatin reduced C-reactive protein-induced NF- κ B activation in human umbilical vein endothelial cells^[156]. Beside NF- κ B, activation of inflammatory transcription factors activator protein 1 and hypoxia-inducible factor 1 α were shown to be down-regulated in human endothelial and vascular smooth muscle cells upon treatment with HMG-CoA reductase inhibitors^[157]. In line with the reported antioxidant and anti-inflammatory properties, statin use has been associated with lower neopterin levels in patients^[158,159].

The influence on redox-balance and Th1-type signalling pathways such as neopterin formation and tryptophan breakdown has been described for a variety of (potentially) cardioprotective antioxidant drugs and vitamins, *e.g.*, aspirin^[160], atorvastatin^[153], vitamins C and E^[161] and seems to be a common mechanism at least *in vitro*. Furthermore, circulating vitamin E was shown to increase upon statin therapy^[162,163]. Thus, due to interferences with common pathways, therapeutic efficacy might change when combining several antioxidant drugs and

supplements.

Furthermore, antioxidant composition may differ between patients, and estimation of antioxidant profiles before therapy could be useful to select candidate patients that would profit from antioxidant therapies^[164,165] and to avoid overdosage. Excessive antioxidant consumption may lead to adverse reactions ranging from favoring of Th2-type responses such as allergy and asthma to an increase of mortality^[166-168]. So far, for patients who respond well to statin/niacin therapy, additional supplementation might only be advantageous when nutritional deficiencies are still detectable, however this hypothesis has to be investigated in more detail.

Another question is, if moderate vitamin deficiencies cannot be better (and safer) regulated by changes of lifestyle factors, *e.g.*, by increasing the consumption of antioxidant-rich food.

NUTRITION, ANTIOXIDANTS AND CVD

The strong relationship between redox-status, immune response and metabolism is supported by the close association of metabolic diseases such as diabetes, obesity and metabolic syndrome with CVD^[169]. Tissues that are important in metabolism are suggested to have an evolutionary potential to mediate inflammatory responses^[170]. Metabolic and immune response pathways are closely cross-regulated to respond to the energetic demands necessary during immune activation. Several metabolic and immune cells show similarities on genetic and functional level, *e.g.*, pre-adipocytes can transdifferentiate into macrophages^[171] and activate similar transcriptional responses^[172].

In contrast to classical activation of the immune system, *e.g.*, by infection or tissue injury, inflammation may also be induced by metabolic triggers. So called metaflammation or para-inflammation is crucially involved in the development of chronic diseases such as diabetes, fatty liver disease and CVD^[172,173].

A variety of dietary factors are able to produce cardiometabolic imprints that predispose to disease development. *E.g.*, increased consumption of trans fatty acids (TFA) is supposed to activate pathways that are linked to insulin resistance syndrome. High TFA intake was found to be associated with harmful changes in serum lipids, systemic inflammation, endothelial function, and prospective observational studies demonstrated strong positive associations with the risk of MI, coronary heart disease death, and sudden death^[174]. Changes of traditional nutrition patterns, as it is the case, *e.g.*, in India, where "Westernization" led to an increase in uptake of sugar, salt, high fat dairy products, and TFA-rich food, are suggested to be at least partially responsible for an about 3-fold increase in the prevalence of CVD and diabetes in the latter part of the 20th century^[175].

But also excessive intake of antioxidants is a burden of modern life due to the omnipresence of preservatives, food colorants and vitamin supplements in the "Western diet". Although still nutritional deficiencies

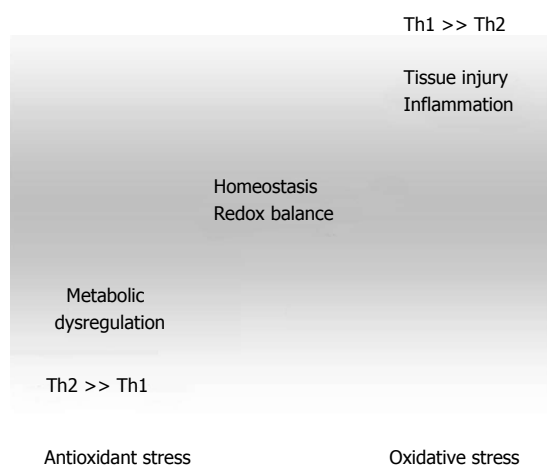


Figure 2 Dysregulation of redox- and Th1/Th2-balance in the course of atherogenesis. Excessive antioxidant intake in combination with other risk factors such as high caloric diet and low physical exercise lead to suppression of Th1-type immunity, thereby favoring Th2-associated development of allergies and asthma and promoting juvenile obesity. Factors such as high blood pressure and hyperlipidemia lead to shear stress and tissue injury. Inflammatory reactions are associated with high reactive oxygen species generation, which results in immunotoxicity due to oxidation of biomolecules (lipids, proteins, etc.).

may exist for some specific vitamins or other antioxidants, overall antioxidant stress may favour a Th2 environment by suppressing Th1 responses (Figure 2). In combination with high caloric diet and low physical activity, this may contribute to the development of obesity^[133]. Food additives such as sodium benzoate, propionic acid, sodium sulfite, sorbic acid and curcumin were shown to suppress Th1-type immune response *in vitro*^[176]. Antioxidant food additives also interfere with satiety saturation circuits, as they have shown to inhibit leptin release in cultured lipopolysaccharide-stimulated murine adipocytes in a dose- and time dependent manner^[177]. Lowering the amount of circulating leptin is suggested to contribute to a obesogenic environment, as the reduced satiety effect in turn could lead to compensatory antioxidant craving and thus even more food intake^[133]. Leptin is considered as a proinflammatory cytokine with proatherogenic features, as it increases monocyte chemoattractant protein-1 and endothelin-1 secretion by endothelial cells, enhances oxidative stress, promotes migration and proliferation of smooth muscle cells and increases platelet aggregation, thus facilitating thrombosis^[178]. In the initial phase of obesity-related inflammation, leptin is predictively associated with interleukin 6 plasma levels in juveniles^[179]. However, leptin resistance, which later develops during obesity, does also favor atherogenesis.

Obesity-related immune mediated systemic inflammation was found to be associated with the development of the metabolic syndrome and altered Trp metabolism. However, across lifespan from juvenility to adulthood, differences in the Trp breakdown rate were observed. While juvenile overweight/obese individuals showed a decreased to unaltered Kyn/Trp ratio in comparison to normal weight controls, obese adults had significantly

elevated Kyn serum levels and an increased Kyn/Trp ratio^[180]. Thus, while in younger patients Th2-type responses might be favored, potentially due to the high antioxidant intake, overwhelming inflammation with Th1-type cytokines may predispose for the development of atherosclerosis in adult age.

Epidemiological observations suggest that consumption of certain foods rich in bioactive compounds, *e.g.*, vitamins E and C, polyphenols and carotenoids such as lycopene and beta-carotene, and coenzyme Q10, is associated with decrease of atherosclerotic risk and such antioxidant-rich diet is supposed to be particularly effective in the early stages of atherosclerosis by preventing LDL oxidation and the oxidative lesion of endothelium^[181,182]. However, a balanced diet cannot always be translated into clinical benefit, despite its beneficial impact on human health.

There is accumulating evidence about the importance of maternal diet and early nutrition on different epigenetic mechanisms that promote the susceptibility to the development of metabolic diseases in adulthood, such as metabolic syndrome, insulin resistance, type 2 diabetes, obesity, dyslipidaemia, hypertension, and also CVD. Of note, both under- and overnutrition have been associated with adverse responses^[183,184]. Several studies indicate that impaired foetal growth, and/or *in utero* exposure to risk factors, especially maternal hypercholesterolaemia, may be relevant for the early onset of cardiovascular damage. Translational studies support this hypothesis; however, a direct causality in humans has not been ascertained^[185].

The influence of epigenetic mechanisms on the developmental induction of chronic diseases raises the possibility that nutritional or pharmaceutical interventions may be used to modify long-term cardio-metabolic disease risk and combat this rapid rise in chronic non-communicable diseases^[186].

CONCLUSION

Adaptive and innate immune responses are centrally involved in the chronic inflammatory process, which leads to destabilization of atherosclerotic lesions, these processes are tightly connected to metabolic factors, which are essentially influenced by life style and also the genetic/epigenetic frame. Inflammation-induced oxidative modifications contribute to all important clinical manifestations of CVD such as endothelial dysfunction and plaque disruption. However, due the poor performance of antioxidant strategies in limiting atherosclerosis and cardiovascular events, it remains to be answered if oxidative modification is causal for the initiation or is an injurious response to atherogenesis^[96]. Disease underlying interactions are too complex and the understanding is too fragmentary that clear, reliable therapeutic recommendations can be given^[101].

The strong interconnection of metabolic and inflammatory pathways suggests that metabolically induced inflammatory processes should be considered as early,

or even primary events^[171]. Many data support that there is a large time span between initial pathological changes and the onset of clinical manifestations. This time frame could be used for preventive strategies, however a better understanding of disease development and more sensitive detection methods would be a prerequisite.

A detailed knowledge on inflammatory and redox-regulated processes would also allow a better adaption of treatment regimes. Stable biochemical markers are necessary to control disease courses and treatment efficacy. In this context, *e.g.*, neopterin is a useful indicator of the immune activation status and oxidative stress^[6] and Kyn/Trp ratio accounts for aspects of immunoregulation *via* IDO and represents an important metabolic checkpoint. Normalization of tryptophan metabolism represents an important goal to improve the outcome of patients suffering from CVD, whereby treatments with IDO inhibitors such as 1-methyl tryptophan could be considered^[101]. However, IDO is well known for its immunosuppressive properties, and its inhibition by medications may also lead to adverse effects.

Also several antioxidant drugs, botanical extracts, phytocompounds and vitamins but also food-contained preservatives and colorants have been shown to negatively interfere with IDO^[87,166]. Both inhibition of enzymatic activity as well as downregulation of activatory signals may lead to a normalization of tryptophan breakdown ratio. Thus, nutrition might be considered as a major factor that influences tryptophan metabolism and underlying inflammation in a more gentle and balanced manner than medication.

Measurement of tryptophan and kynurenine concentrations, and calculation of the Kyn/Trp ratio are important predictors of an unfavourable outcome in patients with CVD. It will be important to investigate if these parameters can provide a basis for more successful and precise biologically grounded therapeutic protocols to further reduce cardiovascular morbidity and mortality^[101]. Combined measurements of multiple markers, such as additional determination of lipoproteins, NO metabolites, BH₄ and plasma antioxidants, will also be helpful to understand redox-regulation in health and disease and may allow to discriminate best between different clinical diagnostic categories and to evaluate treatment strategies.

In summary, a general evaluation of the effect of an “antioxidant therapy” is not possible at the moment. While vitamin supplementation might be beneficial under certain circumstances, a variety of studies indicate no or even adverse effects when administered alone and even more when used in combination with lipid-lowering agents. However, also for statin and niacin treatment a panel of adverse effects has been described^[187,188]. Although antioxidant supplementation may have some benefit to counteract secondary symptoms, their role in CAD seems to be of moderate importance^[145]. Surveillance of the antioxidant status before and during therapy would allow seek out patients that could benefit from vitamin supplementation^[164,165]. Impact of lifestyle factors such as nutrition and physical exercise, however, has turned out

as a major factor in CVD prevention and also in influencing treatment efficacy.

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Cardiomyopathies: Evolution of pathogenesis concepts and potential for new therapies

Hamayak Sisakian

Hamayak Sisakian, Department Cardiology, Clinic of General and Invasive Cardiology, University Hospital 1, Yerevan State Medical University, Yerevan 0025, Armenia

Author contributions: Sisakian H solely contributed to this paper.

Correspondence to: Hamayak Sisakian, Professor, Department Cardiology, Clinic of General and Invasive Cardiology, University Hospital 1, Yerevan State Medical University, 2 Koryun street, Yerevan 0025, Armenia. hamayak_sisakyan@hotmail.com

Telephone: +374-10-582023 Fax: +374-10-541350

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Abstract

Cardiomyopathies are defined as diseases of the myocardium with associated structural and functional abnormalities. Knowledge of these pathologies for a long period was not clear in clinical practice due to uncertainties regarding definition, classification and clinical diagnosis. In recent decades, major advances have been made in the understanding of the molecular and genetic issues, pathophysiology, and clinical and radiological assessment of the diseases. Progress has been made also in management of several types of cardiomyopathy. Advances in the understanding of these diseases show that cardiomyopathies represent complex entities. Here, special attention is given to evolution of classification of cardiomyopathies, with the aim of assisting clinicians to look beyond schematic diagnostic labels in order to achieve more specific diagnosis. Knowledge of the genotype of cardiomyopathies has changed the pathophysiological understanding of their etiology and clinical course, and has become more important in clinical practice for diagnosis and prevention of cardiomyopathies. New approaches for clinical and prognostic assessment are provided based on contemporary molecular mechanisms of contribution in the pathogenesis of cardiomyopathies. The genotype-phenotype

complex approach for assessment improves the clinical evaluation and management strategies of these pathologies. The review covers also the important role of imaging methods, particularly echocardiography, and cardiac magnetic resonance imaging in the evaluation of different types of cardiomyopathies. In summary, this review provides complex presentation of current state of cardiomyopathies from genetics to management aspects for cardiovascular specialists.

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Key words: Dilated cardiomyopathy; Hypertrophic cardiomyopathy; Restrictive cardiomyopathy; Arrhythmic cardiomyopathy; Secondary cardiomyopathy

Core tip: Cardiomyopathies represent a different group of disorders in which myocardium is itself structurally and functionally abnormal. During recent decades, the genetics, pathophysiology and diagnosis of cardiomyopathy have advanced from the traditional methods of clinical presentation to new genetic and imaging techniques. Nevertheless, the differences in definition, classification, pathophysiological mechanisms and diagnosis are controversial issues in clinical practice. The purpose of this review is to present the current state of classification, genetics, diagnostic approaches and management in order to provide useful instructions for clinical practice.

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INTRODUCTION

Cardiomyopathies are defined as myocardial disorders in

Table 1 American Heart Association classification for cardiomyopathies

Primary cardiomyopathies	Genetic	HCM/ ARVC/LVNC/Conduction defects/Mitochondrial myopathies/ion channel disorders
	Mixed	DCM/restrictive
Secondary cardiomyopathies	Acquired	Inflammatory/Tako-Tsubo/Peripartum/Tachycardia induced/Infants of IDDM mothers
	Infiltrative	Amyloidosis, Gauchers, Hurler's, Hunter's
	Storage	Fabry's, Glycogen storage disease, Niemann-Pick disease, haemochromatosis
	Toxicity	Drugs, heavy metals
	Endomyocardial	EMF, Loeffler's endocarditis
	Inflammatory	Sarcoidosis
	Endocrine	Diabetes, hyperthyroidism, hypothyroidism, hyperparathyroidism
	Cardiofacial	Noonan's, lentiginosis
	Neuromuscular	Friedreich's ataxia, Duchenne-Becker muscular dystrophy, myotonic dystrophy
	Nutritional	Beriberi, scurvy, selenium
	Autoimmune	SLE, dermatomyositis, scleroderma
	Consequence of cancer therapy	Anthracyclines, radiation, cyclophosphamide,

ARVC: Arrhythmogenic right ventricular cardiomyopathy/dysplasia; DCM: Dilated cardiomyopathy; HCM: Hypertrophic cardiomyopathy; LVNC: Left ventricular non-compaction; EMF: Endomyocardial fibrosis.

which the myocardium is structurally and/or functionally abnormal in the absence of definite disease able to cause the myocardial pathology. Cardiomyopathies are classified traditionally according to morphological and functional criteria into four categories: dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM) and arrhythmogenic right ventricular (RV) cardiomyopathy/dysplasia (ARVC/D). DCM is the most common form of heart muscle disease, comprising approximately 60% of all cardiomyopathies and characterized by left ventricular (LV) dilation and systolic dysfunction. The dilated cardiomyopathy is often assumed as a common pathway of several cardiovascular pathologies.

EVOLUTION OF CLASSIFICATIONS

Cardiomyopathies are classified as either primary or secondary. Primary cardiomyopathies consist of disorders namely or predominantly confined to the heart muscle, which have genetic, nongenetic, or acquired causes. Secondary cardiomyopathies are disorders that have myocardial damage as a result of systemic or multi-organ disease^[1]. These cardiomyopathies can be primary myocardial disorders or develop as a secondary consequence of a variety of conditions, including myocardial ischemia, inflammation, infection, increased myocardial pressure or volume load and toxic agents.

In the 1980 World Health Organization (WHO) classification, cardiomyopathies were classified as "heart muscle diseases of unknown cause", reflecting a general lack of etiologic factors which may cause heart failure. The next WHO classification published in 1995 proposed "diseases of myocardium associated with cardiac dysfunction" and included for the first time ARVC/D, as well as primary RCM^[2,3].

A more recent definition and classification of cardiomyopathies was proposed by the American Heart Association (AHA) Scientific Statement Panel, which divides cardiomyopathies as follows: "Cardiomyopathies are a heterogeneous group of diseases of the myocardium as-

sociated with mechanical and/or electrical dysfunction, which usually (but not invariably) exhibit inappropriate ventricular hypertrophy or dilatation, due to a variety of etiologies that frequently are genetic. Cardiomyopathies are either confined to the heart or are part of generalized systemic disorders, and often lead to cardiovascular death or progressive heart failure-related disability"^[1].

So far as the classification of cardiomyopathies is difficult, because the etiology or pathophysiology is not always clarified, there is no agreement on classification approaches in regular clinical practice.

For promoting standard nomenclature, recent knowledge on underlying causes and pathophysiology of cardiomyopathies has been implemented in a cardiomyopathy classification system both on behalf of the AHA and European Society of Cardiology (ESC)^[4].

The AHA divides cardiomyopathies into two major groups based on predominant organ involvement. Primary cardiomyopathies (genetic, nongenetic, or acquired) are those solely or predominantly confined to heart muscle and are relatively less common. Secondary cardiomyopathies show pathological myocardial involvement as part of a several number of systemic pathologies (Table 1)^[5].

In 2013, the MOGE(S) classification for a phenotype-genotype nomenclature of cardiomyopathies was proposed by the World Heart Federation^[6]. This classification suggests a nosology that addresses five characteristics of cardiomyopathic disorders: morphofunctional state (M), organ involvement (O), genetic inheritance (G), etiologic annotation (E) and functional state (S) according to ACC/AHA A-D stage and New York Heart Association (NYHA) I-IV functional class. The description of five characteristics provides classification in MOGE(S) designation. The MOGE(S) classification has several advantages with regard to simultaneous maximal description of disease from clinical and genetic points. However, this classification does not fulfill the diagnostic criteria of cardiomyopathies in several clinical situations and may not be always applied in clinical practice, because of the lack of genetic testing in many clinical centers. On the other hand, the classification based on systematically genetic

testing and monitoring may cause overdiagnostic states without clinically evident signs of cardiomyopathies and absence of clinical phenotype. Further genetic research and development of multicenter registries are needed to clarify the clinical advantages and to make more practical of MOGE(S) classification of cardiomyopathies.

DCM

DCM represents the most common cardiomyopathy worldwide. It is a heart muscle disorder defined by the presence of a dilated and poorly functioning left or both ventricles. It can be primary (genetic, mixed or predominantly familial nongenetic, or acquired) or secondary (inflammatory, autoimmune, or thyrotoxic). This disease can be diagnosed in association with recognized cardiovascular disease; however, to qualify as DCM, the extent of myocardial dysfunction cannot be explained exclusively by abnormal loading conditions (hypertension or valve disease) or ischemic heart disease^[4,7]. A large number of cardiac and systemic diseases can cause systolic dysfunction and LV dilatation, but in the majority of cases no definite cause is found. This has led to the common terminology idiopathic dilated cardiomyopathy (IDC).

PREVALENCE

Prevalence in the general population remains undefined. This disorder develops at any age, in either sex, and in people of any ethnic origin^[8,9]. In adults, DCM arises more commonly in men than in women. In children, the yearly incidence is 0.57 cases per 100000, but is higher in boys than in girls (0.66 *vs* 0.47 cases per 100000, *P* < 0.006). Two-thirds of children are thought to have idiopathic disease^[4]. In adults, the prevalence is 1 in 2500 individuals, with an incidence of 7 per 100000 per year (but it could be underdiagnosed). The prevalence of DCM in the United States (adjusted for age) is 36 per 100000 of the population^[8]. The etiology includes genetic transmission (estimated at 30%-40%) identifying familial DCM, cytotoxic agents (*e.g.*, anthracycline derivatives), malnutrition (*e.g.*, protein deficiency), myocarditis (viral etiology), and autoimmune disease. In many cases, the disease is inherited, and is called familial dilated cardiomyopathy (FDC). The familial type might account for 20%-48% of all cases^[10].

FAMILIAL (GENETIC) DILATED CARDIOMYOPATHY

Prominent progress has been made in studies of the genetics of DCM. Most of the genes involved in the development of DCM encode structural elements of the cardiomyocytes, particularly dystrophy associated glycoprotein complex or components of the sarcomeric complex. Genetic predisposition may have a decisive role in the development of primary and secondary DCM. Currently, > 30 autosomal and 2-X-linked genes have been

shown to predispose to DCM and the number of these genes will continue to increase. There are sufficient data that with new diagnosis of IDC the clinical screening of first-degree family members will reveal familial (genetic) DCM in 20%-35% of those family members. Recent guidelines recommend that genetic testing should be provided in families in whom familial DCM is suspected for early diagnosis of cardiomyopathy in family members^[4].

The diagnosis of FDC is made when IDC is diagnosed in two closely related family members. About 20%-48% of DCM has been reported as familial, although with incomplete and age-dependent penetrance, and linked to a diverse group of > 20 loci and genes^[10]. Although genetically heterogeneous, the predominant mode of inheritance for DCM is autosomal dominant, with X-linked autosomal recessive and mitochondrial inheritance being less frequent. Thus, when taking a family history, specific attention should be given to a history of muscular dystrophy, features of mitochondrial disease (*e.g.*, familial diabetes, deafness, epilepsy, or maternal inheritance), and signs and symptoms of other inherited metabolic diseases^[10]. Several of the mutant genes linked to autosomal dominant DCM encode the same contractile sarcomeric proteins that are responsible for HCM, including α -cardiac actin; α -tropomyosin; cardiac troponin T, I and C; β - and α -myosin heavy chain; and myosin binding protein C. Z-disc protein-encoding genes, including muscle LIM protein, α -actinin-2, ZASP, and titin, also have been identified. DCM is also caused by a number of mutations in other genes encoding cytoskeletal/sarcomeric, nuclear envelope, sarcomere, and transcriptional coactivator proteins. The most common of these probably is the lamin A/C gene, also associated with conduction system disease, which encodes a nuclear envelope intermediate filament protein. Mutations in this gene also cause Emery-Dreifuss muscular dystrophy^[11-13]. Other DCM genes of this type include desmin, caveolin, and β - and α -sarcoglycan, as well as the mitochondrial respiratory chain gene^[1]. X-linked DCM is caused by the Duchenne muscular dystrophy (dystrophin) gene, whereas G4.5 (tafazzin), a mitochondrial protein of unknown function, causes Barth syndrome, which is an X-linked cardioskeletal myopathy^[10,13].

PATHOLOGY

Macroscopic examination

Macroscopic examination of heart reveals ventricular chamber dilation with thickened or normal thickness walls. Valvular changes are not typical, although dilation of valvular orifices may be present as secondary changes due to dilation of chambers. Coronary anatomy is most commonly normal, although the presence of nonocclusive atherosclerotic plaques may be present. Thrombi are found most frequently in ventricles and atrial appendages.

Histological examination

The most typical DCM pattern is the development of

interstitial and perivascular fibrosis of varying degree^[14]. Myocardial necrosis predominantly is present at subendocardium. Our study group investigated noninvasively using the Shirani method^[15] the degree of myocardial fibrosis in patients with IDC and ischemic dilatation cardiomyopathy. The percentage of volumic collagen fraction in the LV myocardium was significantly higher in DCM patients compared to those with ischemic cardiomyopathy. Increase of collagen fraction correlated with the degree of dilation of the left ventricle^[16].

Clinical manifestations

The most common clinical manifestations of DCM are congestive heart failure symptoms and thromboembolic complications. The disease commonly has a progressive course. The determination of time of manifestation is not easy, because the disease course for a long period is not symptomatic. Patients are admitted to hospital in cases with expressed heart failure symptoms. A careful history taking and physical examination with diagnostic studies are essential for differential diagnosis of DCM. More commonly, DCM manifests without any history and provoking factor. Cardiomegaly at radiological examination or on abnormal electrocardiography (ECG) may be the first findings in an asymptomatic patient. The left ventricle is dilated, and more spherical than usual with raised wall stress and depressed systolic function. As the disease progresses, definite symptoms of congestive heart failure present. Chest discomfort may occur in some cases, however this discomfort is not relieved by nitroglycerin. Physical examination may reveal gallop rhythm in decompensated patients. The jugular venous pulse is normal until right heart decompensation is present. The clinical course of DCM may be variable both with slow progression and rapidly progressive over several months. Cachexia and peripheral edema typically arise late in the course. Sudden death, presumably due to ventricular fibrillation may be the first manifestation. Some cases of DCM most probably develop due to viral myocarditis and these patients may have a history viral infection prior to deterioration of heart failure symptoms. An acute systemic febrile infectious disease (such as influenza) is followed by a latent period during which time the patient may be asymptomatic. It is reported also that in 20%-25% of patients with new-onset DCM may have cardiac recovery^[17].

Several clinical, laboratory and instrumental factors may have prognostic significance in DCM patients. These factors are symptomatic ventricular arrhythmias, persistent gallop rhythm, persistent jugular venous distention, systemic hypotension, persistently elevated B-type natriuretic peptide, left bundle branch block, pulmonary capillary wedge pressure > 20 mmHg, cardiac index < 2.5 L/min per square meter, severely reduced ejection fraction, restrictive diastolic filling pattern, and severe mitral regurgitation^[18].

ECG

ECG in patients with idiopathic DCM has no specific

diagnostic role, and abnormalities ranging from isolated T wave and ST segment changes to septal pathological Q waves, wide QRS complex in patients with LV fibrosis might be present. Prolongation of atrioventricular (AV) conduction, and bundle branch block can be observed. Sinus tachycardia and supraventricular arrhythmias are common, in particular atrial fibrillation. Approximately, 20%-30% of patients have nonsustained ventricular tachycardia and a small number present with sustained ventricular tachycardia. ECG is utilized as a first-line screening and diagnostic tool for detecting conditions associated with sudden death. Idiopathic DCM patients with a prolonged QRS have significantly worse survival than other patients^[19].

ECHOCARDIOGRAPHY

Echocardiography in DCM has characteristic patterns, although it is not possible to make differential diagnosis by echocardiography between idiopathic and other secondary LV dilation with dysfunction. M-mode echocardiography shows LV dilation with diffuse hypokinetic walls (Figure 1). Although cardiomyopathy is diffuse pathology, there may be segmental differences of the degree of hypokinesis revealed by two-dimensional echocardiography, which causes difficulties for differentiation from ischemic cardiomyopathy. Ventricular dilation usually is not accompanied by sufficient hypertrophy, which causes increase of volume-to-mass ratio^[20]. Doppler echocardiography shows frequently functional mitral and tricuspid regurgitation and a different degree of diastolic dysfunction, depending on severity of intracardial hemodynamic abnormalities.

CARDIAC CATHETERIZATION

Catheterization for exclusion of coronary artery disease is important for following management of DCM patients. Catheterization also may reveal increased LV end-diastolic pressure and pulmonary artery wedge pressure. Left ventriculography may show ventricular dilation with global hypokinesis.

CARDIAC MAGNETIC RESONANCE IMAGING AND DILATED CARDIOMYOPATHY

Cardiac magnetic resonance imaging (MRI) can differentiate ischemic from non-ischemic cardiomyopathies through use of late gadolinium imaging, even when the heart is globally dilated and dysfunctional (Figure 2). Infarction is characteristic in that it always causes subendocardial late gadolinium enhancement (LGE), which extends variably transmurally to the epicardium. It also follows a coronary territory distribution. The absence of LGE in a dysfunctional segment of myocardium implies the potential for recovery with time (stunning), medical treatment or revascularization (hibernation), biventricular pacing (dyssynchrony)^[21]. Nonischemic DCM may dem-

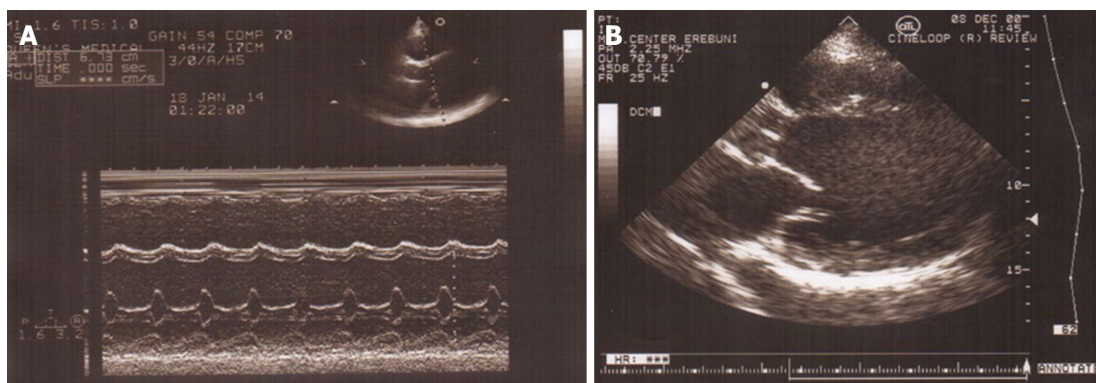


Figure 1 M mode and B mode echocardiogram of patient with idiopathic dilated cardiomyopathy. A: M mode echocardiogram shows dilated left ventricle with hypokinesis of interventricular septum and posterior wall; B: Parasternal long axis view of B mode echocardiogram showing remodeled left ventricular shape with loss of elliptical form.

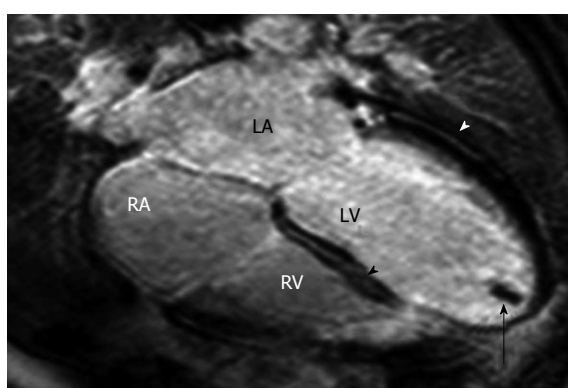


Figure 2 Dilated cardiomyopathy in a 36-year-old male soccer player with fatigue and a 3-5-d history of burning epigastric pain associated with nausea, vomiting, and early satiety^[105]. Horizontal long-axis late contrast-enhanced magnetic resonance imaging shows an apical thrombus (arrow) in the left ventricle (LV) and midwall enhancement in the lateral left ventricular wall (white arrowhead) and the interventricular septum (black arrowhead). RA: Right atrium; RV: Right ventricle; LA: Left atrium.

onstrate either no LGE or mid-wall LGE in areas not corresponding to a coronary territory. Additional features that can be detected using cardiac MRI include valvular regurgitation, apical thrombus, dyssynchrony with or without posterior scar, signs of decompensation, cardiac iron, LV hypertrophy (LVH), RV involvement and atrial size.

CHRONIC MYOCARDITIS AND DCM

The major long-term consequence of myocarditis is inflammatory dilated cardiomyopathy, but the pathways that lead to myocardial fibrosis are poorly understood.

The gold standard of diagnosing the underlying causes of myocarditis and inflammatory cardiomyopathy is the histological, immunohistological and polymerase chain reaction-based analysis of cardiac MRI-guided endomyocardial biopsy (EMB) specimens. Persistent viral infections and infection-associated or postinfectious inflammatory processes of the myocardium may be key pathological mechanisms of progression of myocarditis to cardiomyopathy.

ANTIVIRAL THERAPY APPROACH

Several recent studies have investigated endomyocardium-based etiological antiviral treatment of inflammatory cardiomyopathies.

Interferons serve as a natural defense against many viral infections. Their innate production is associated with clinical recovery from viral infection and subsequent sequelae, while exogenous administration is protective. Type I interferons are a promising choice for treatment of chronic viral myocarditis. Currently, there is no approved treatment for chronic viral heart disease, but data from open-label phase II studies have demonstrated that subgroups of patients, who had not improved upon regular heart failure medication, may have significant benefit even years after onset of chronic disease. In the study of a 6-mo interferon- β 1a therapy of patients with persistent enteroviral and adenoviral myocarditis, complete elimination of enteroviral and adenoviral genomes was demonstrated by follow-up biopsies taken 3 mo after termination of antiviral therapy. Virus clearance was paralleled by an improvement of mean LV function, a decrease in ventricular size, amelioration of heart failure symptoms, and a decrease of infiltrating inflammatory cells. No patient deteriorated and patients with severely affected LV dysfunction gained most benefit. Viral elimination after antiviral treatment suggests that early biopsy-based diagnosis and timely treatment may prevent disease progression and thereby improve the outcome of chronic viral cardiomyopathy. However there are limited data on efficacy of specific antiviral therapies and more studies are needed to identify patient cohorts who will benefit from targeted antiviral or immunosuppressive therapy. Treatment of myocarditis in current regular clinical practice remains supportive including the need for ventricular assist devices and heart transplantation^[22].

EMB

In recent years, EMB has become a useful diagnostic tool for the investigation and treatment of myocardial diseases. However, its routine use is criticized by some

authors for the lack of therapeutic usefulness^[23]. The techniques enable us to obtain multiple tissue samples from both ventricles with a low incidence of procedural complications. In addition to several clinical states such as after heart transplantation, specific myocardial diseases, the more frequent indication for EMB is suspected myocarditis in patients with progressive heart failure. In such cases, the correct analysis of tissue samples represents an important point to diagnosis. Although EMB provides suggestive findings in DCM, these findings may not always be revealed due to the technical difficulties of procedure and biopsy specimens may not contain pathological changes. The diagnostic performance of EMB is superior if the procedure is provided with a cardiac MRI-guided target area^[24]. Diagnostic findings that show absence of inflammation may assist in further management strategies for DCM. Thus, in selected cases, EMB represents a useful method for correct prognostic and therapeutic evaluation of DCM.

MANAGEMENT

There is no specific etiology-based therapy in DCM. The main principles of DCM treatment are general concepts of chronic heart failure treatment. Although conventional pharmacotherapy is not specific with regard to etiopathogenesis, it decreases mortality in such patients. Common treatment includes β -blockers, angiotensin-converting enzyme (ACE) inhibitors, spironolactone in patients with NYHA class II-IV heart failure. Diuretic therapy may have a beneficial effect on symptoms without a prominent effect on long-term outcome. β -Blockers and amiodarone can be used for management of supraventricular and ventricular arrhythmia. However, their long-term effect did not reduce mortality conditioned by sudden cardiac death (SCD)^[25]. An implantable cardioverter defibrillator (ICD) and biventricular pacemakers are indicated in appropriate patients with both idiopathic and secondary dilated cardiomyopathies with LV dysfunction for secondary prevention of SCD. ICD can be combined with cardiac resynchronization therapy in patients with prolonged QRS duration and LV dys-synchrony^[26]. However, the benefits of ICD were established in patients with systolic dysfunction of ischemic etiology^[25,27]. Individual studies in patients with nonischemic cardiomyopathy failed to show significant reduction of total mortality^[28-30], although a meta-analysis of five trials showed 31% mortality reduction^[31].

Surgical approaches to restore LV shape by reverse remodeling include LV reconstruction and implantation of external restraint devices. The aims of ventricular reconstruction procedures are to restore elliptical ventricular chamber to decrease wall stress, end systolic volume and mitral regurgitation^[32]. Most of these reconstruction procedures and trials have been estimated in patients with ischemic origin DCM.

The selected ventriculoplasty in combination with mitral annuloplasty is a useful option for patients with an extremely dilated left ventricle in IDC. Surgery should

be considered before inotropic dependency occurs when prior medical treatment has failed^[33].

In carefully selected patients, partial ventriculectomy combined with mitral valve reconstruction achieves short-term results comparable to those after heart transplantation^[34]. However, long-term results and multicenter evaluation are needed to define its place in the treatment of advanced heart failure. With studies directed to patient selection and surgical modification, ventriculoplasty will become a realistic option in the treatment of heart failure caused by nonischemic cardiomyopathy.

Stem cell therapy has shown moderate effects in clinical trials for ischemic cardiomyopathy, but it remains to be determined if these results are applicable to idiopathic DCM patients. There is a need for methodologically sound studies to elucidate underlying mechanisms and translate those into improved therapy for clinical practice. In a single center study with 110 patients with nonischemic DCM, intracoronary CD34⁺ stem cell transplantation was associated with improved ventricular function, exercise tolerance, and long-term survival^[35]. Higher intramyocardial homing in this study was associated with better stem cell therapy response.

To prove safety and efficacy of cell therapy for DCM, adequate randomized (placebo) controlled trials using different strategies are mandatory. The REGENERATE-DCM trial is the first ongoing randomized, double-blind, placebo-controlled trial worldwide to investigate the role of granulocyte-colony-stimulating factor and autologous bone-marrow-derived stem/progenitor cell therapy to improve cardiac function in patients with DCM^[36].

The 5-year survival averages 30%-40% and is improved by contemporary heart failure therapy, but not all patients respond well to therapy and some patients rapidly deteriorate no matter the therapeutic approach, and for them, heart transplantation remains the only option.

CARDIOMYOPATHIES WITH DILATED PHENOTYPE

Peripartum cardiomyopathy

Peripartum cardiomyopathy (PPCM) is a rare but potentially life-threatening condition that occurs in previously healthy women during the last month of pregnancy and up to 5-6 mo postpartum. The etiology and pathophysiology remain uncertain, although recent observations strongly suggest the specific role of prolactin cleavage secondary to unbalanced peri-/postpartum oxidative stress^[37]. PPCM is a diagnosis of exclusion, because it shares many clinical characteristics with other forms of systolic heart failure secondary to cardiomyopathy. The heart failure management requires a multidisciplinary approach during pregnancy, considering the possible adverse effects on the fetus. Some novel therapies, such as prolactin blockade, are proposed to either prevent or treat the patients with PPCM^[38]. A critical individual approach concerning the risks of subsequent pregnancy must be considered. As a result of its rare incidence, geographical

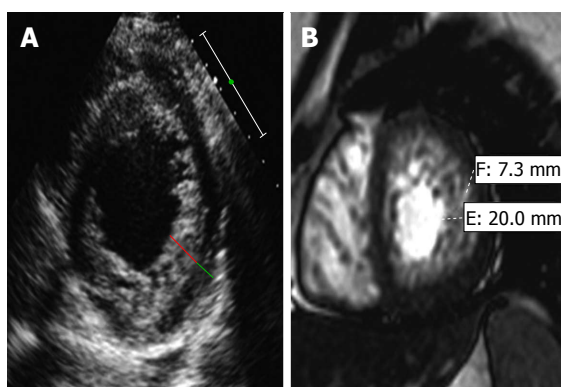


Figure 3 Non-compaction cardiomyopathy in two patients^[105]. A: Dilated cardiomyopathy in a 60-year-old man with new-onset congestive heart failure. Short-axis echocardiogram obtained in systole at the level of the left ventricle (LV) shows a two-layered myocardium with a noncompacted (red line) and compacted (green line) layer along the lateral, inferior, and anterior walls and a maximal end-systolic NC: C ratio > 2 ; B: Symptoms of New York Heart Association Class III heart failure and severely reduced ($\leq 35\%$) LV ejection fraction in a 35-year-old woman. Short-axis 2D SSFP cardiac magnetic resonance imaging obtained in end diastole shows thickening of the LV myocardium, with an NC: C ratio of 2.9. The patient underwent subsequent implantable cardioverter-defibrillator placement for primary prevention of sudden cardiac death.

differences, and heterogeneous presentation, PPCM continues to be incompletely characterized and understood. For all these reasons, PPCM remains a challenge in clinical practice, so future epidemiological trials and national registries are needed to learn more about the disease.

Classic criteria of PPCM include development of heart failure in the last month of pregnancy or within the first 5 mo postpartum, absence of an identifiable cause of heart failure, and absence of recognizable heart disease prior to the last month of pregnancy^[39].

LV non-compaction

LV non-compaction (LVNC) is a cardiomyopathy resulting from arrest of fetal development of the heart. This leads to altered myocardial architecture that is seen as a two-layered myocardium with a thin, compacted epicardial layer and a thick, noncompacted endocardial region. The noncompacted myocardial region is comprised of prominent trabeculations and deep intertrabecular recesses that directly communicate with the LV cavity. The condition may present without any associated cardiac malformation and is then labeled isolated LVNC. Non-compacted myocardium is also seen in conjunction with other cardiac abnormalities including cyanotic congenital heart disease, Ebstein's anomaly, and other cardiomyopathies. Clinical presentation in LVNC is seen with congestive heart failure, ventricular arrhythmia, and systemic thromboembolism. The condition is listed as an unclassified cardiomyopathy in the WHO and ESC classification of cardiomyopathies^[4] and as a primary genetic cardiomyopathy in the AHA classification^[5].

Both sporadic and familial forms are described.

The presence of significant non-compaction is estimated at 1:2000 in the general population. The condition

is, however, more prevalent in heart failure patients. More frequent use of cardiac imaging in clinical practice has increased recognition of this condition^[40].

Non-compaction myocardium clinically may represent from asymptomatic individuals to those with severe disease presenting with heart failure, ventricular arrhythmia, and systemic thromboembolism. Noncardiac features may include facial dysmorphism and neuromuscular disorders.

Echocardiography may reveal trabeculation in the LV wall. However in healthy persons this can be also found. To separate benign LV trabeculation from pathological LVNC following diagnostic criteria is proposed^[41].

Echo: Ratio of noncompacted to compacted myocardium in end-systole of $> 2:1$.

Cardiac MRI: Ratio of noncompacted to compacted myocardium in end-diastole of $> 2.3:1$. Cardiovascular imaging is important in the diagnosis of LV non-compaction. Cardiac MRI (Figure 3) has better resolution compared to echocardiography, which makes it a preferred imaging modality in such patients. Cardiac MRI is also reliable in distinguishing LVNC from other causes of LV apical deformity, including apical variant of hypertrophic cardiomyopathy, endomyocardial fibrosis (EMF) and apical thrombus^[42]. Pharmacological management of LVNC is mainly symptomatic and directed to relief of heart failure symptoms. Heart transplantation remains an option in patients with treatment-tolerant high functional class patients. Ventricular arrhythmia is not directly related to severity of LV dysfunction and a prophylactic ICD is recommended. Anticoagulation to prevent thromboembolic complications is recommended, particularly in patients with severe contractile dysfunction.

STRESS-INDUCED OR "TAKOTSUBO" CARDIOMYOPATHY

Stress-induced cardiomyopathy was termed Takotsubo cardiomyopathy by Japanese cardiologists in 1991^[43]. Advances in diagnostic imaging and emergency coronary angiography have contributed to increased recognition of stress-induced cardiomyopathy, and increasing numbers of reports have been published since then.

A history of intense emotional or physical stress and a typical pattern of LV contractile dysfunction on cardiac imaging are suggestive of the diagnosis. The most common abnormality on ECG is ST-segment elevation resembling ST segment elevation myocardial infarction^[44]. This cardiomyopathy is transient and reversible. Clinical presentation may be indistinguishable from acute coronary syndrome, invariably necessitating coronary angiography for exclusion of obstructive coronary artery disease. Prevalence is in 1%-2% of patients undergoing coronary angiography for acute coronary syndrome. Based on morphological features of the LV, presumed causative role of stress and catecholamine excess and transient nature of the contractile dysfunction, other

nomenclature used to describe this cardiomyopathy include ampulla cardiomyopathy, stress cardiomyopathy or catecholamine cardiotoxicity and transient LV apical ballooning syndrome^[45].

Distinct pattern of contractile abnormality is noted in the left ventricle. In the typical case the LV apex is dyskinetic and expanded and may be associated with hyperdynamic contractility of the basal LV segments. The shape of left ventricle in systole resembles a Japanese octopus trap (Takotsubo), which has a narrow neck and a wide base. The condition is associated with markedly elevated circulating catecholamine, which is assumed to be central in the pathophysiology of this condition though exact mechanism at the cellular level is not fully understood. In a report by Wittstein *et al*^[46], two to three times higher plasma catecholamine concentrations were found in 13 patients with transient LV apical ballooning syndrome compared with seven controls hospitalized for acute myocardial infarction with Killip class III heart failure. Preponderance of females afflicted by this condition is unclear.

Estrogen deficiency in the postmenopausal state may play a role^[47]. Of particular interest, in other conditions with elevated catecholamine levels like subarachnoid hemorrhage, segmental wall motion abnormality is also predominantly seen in women. A reverse pattern of contractile abnormality with apical sparing has also been reported. Cardiac MRI is helpful in diagnosing and monitoring clinical recovery. Absence of delayed hyperenhancement on cardiac MRI is particularly important in differentiating this condition from ischemic and other types of nonischemic cardiomyopathy and acute myocarditis: normal first-pass contrast enhanced rest myocardial perfusion, reversible myocardial edema in regions of contractile dysfunction, and absence of late gadolinium enhancement is strongly indicative of the diagnosis of Takotsubo cardiomyopathy. Resolution of contractile dysfunction, days to weeks after initial presentation, is confirmatory of the diagnosis.

DRUG-INDUCED CARDIOMYOPATHIES

Several drugs may cause acute and chronic cardiac systolic dysfunction with the development of myocardial remodeling. Many of drugs administered chronically are cardiotoxic and may trigger the development of cardiac injury even when used appropriately. ESC guidelines emphasize some specific drug groups, which are strongly related to development of heart failure^[48].

Anthracyclines are highly effective antineoplastic agents with wide application. However, one of the major complications in their long-term pharmacotherapy is cardiac dysfunction. Three distinct types of anthracycline-induced cardiotoxicity have been described^[49]. Acute or subacute injury can occur immediately after treatment with transient arrhythmias, pericarditis and myocarditis. These manifestations usually respond rapidly with interruption of anthracycline infusion. Long-term therapy may be associated with chronic cardiotoxicity resulting in cardiomyopathy. Late-onset anthracycline cardiotoxicity

may cause ventricular dysfunction and arrhythmias, which manifest years to decades after anthracycline treatment has been completed.

Echocardiography may serve as excellent diagnostic tool both for diagnosing and for screening, monitoring of patients on antineoplastic therapy.

A clinical study estimating the cumulative percentage of patients who developed doxorubicin-induced congestive heart failure found that cumulative dose of 400 mg/m² was 3%, increasing to 7% at 550 mg/m² and to 18% at 700 mg/m². Current anthracycline regimens typically contain less than the cumulative dose associated with increased risk of cardiomyopathy^[50,51].

Standard treatment for systolic heart failure is indicated for treatment for both asymptomatic and symptomatic cases, with ACE inhibitors, β -blockers, spironolactone.

Several agents have been studied to decrease cardiotoxicity in such patients. Dexrazoxane (also known as cardioxane) is the most investigated agent^[52,53]. It is the only approved cardioprotective agent in anthracycline chemotherapy, but there is no evidence for a difference in response rate or survival^[54]. Other agents such L-carnitine, coenzyme Q10, N-acetylcysteine, vitamin E, and trimetazidine, have been investigated as metabolic cardioprotective agents^[55-62]. Unfortunately, none of them showed prominent clinical efficacy in preventing anthracycline toxicity.

The alkylating agent cyclophosphamide is mainly cardiotoxic at high doses in bone marrow transplantation protocols^[63]. Cardiotoxicity is expressed from transient electrocardiographic changes and asymptomatic increases of serum levels of cardiac enzymes to severe cardiotoxicity such as exudative pericardial effusion, ventricular hypertrophy and fatal myopericarditis and (hemorrhagic) myocardial necrosis^[64].

ALCOHOLIC CARDIOMYOPATHY

Alcoholic cardiomyopathy represents one of the most common forms of secondary cardiomyopathies resembling IDC. The risk of development of alcoholic cardiomyopathy depends on both duration and doses of alcohol consumption. The clinical course and prognosis in alcoholic cardiomyopathy in withdrawal of alcohol consumption is better compared to those with idiopathic DCM^[65,66]. The diagnosis of alcoholic cardiomyopathy may have several difficulties with regard to widespread consumption of alcohol in many countries, including patients with idiopathic DCM and similarities of radiological patterns of myocardial remodeling in both idiopathic and alcoholic cardiomyopathy^[67].

ARRHYTHMOGENIC CARDIOMYOPATHY

Arrhythmogenic cardiomyopathy/RV dysplasia is the genetic form of cardiomyopathy characterized by fibrosis and fatty infiltration of RV myocardium and by manifestation of ventricular tachycardia/ventricular fibrillation. Lately, it has been shown that the disease is not confined

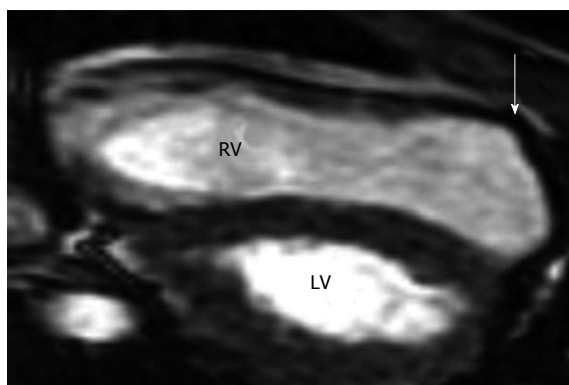


Figure 4 Arrhythmogenic right ventricle cardiomyopathy in a 17-year-old boy who experienced sudden cardiac death from sustained ventricular tachycardia during a soccer match and was revived with on-site defibrillation^[105]. Parasternal long-axis 2D echocardiograms obtained in end systole show a dilated right ventricle (RV) and regional dyskinesia at the RV outlet tract (arrow). LV: left ventricle.

only to the right ventricle as the name suggests, because the left ventricle may be affected in up to 75% of patients^[68]. This disease accounts for 20% of cases of SCD and mainly among young athletes dying suddenly, the prevalence of this cardiomyopathy is higher. In 30%-50% of cases arrhythmogenic cardiomyopathy represents family disease with autosomal-dominant inheritance of gene mutations encoding desmosomal proteins^[69]. Presenting symptoms range from palpitation to syncope and SCD. Myocardial electrical instability comprises the main clinical manifestation with ventricular ectopics and ventricular tachycardia. Biventricular or RV failure is less common and observed mainly in patients with long-term disease protected from SCD by ICD implantation.

Diagnosis of this condition may cause difficulties with nonspecific abnormalities on echocardiographic and angiographic examinations. EMB has a low sensitivity, because samples are usually taken from the septum; a region that is infrequently involved^[70]. ECG may have a diagnostic role with the following typical characteristics: wide QRS complexes in right chest leads, T wave inversion, and ϵ wave after QRS complex as a prototype of late ventricular potentials. The task force determined diagnostic criteria for arrhythmogenic cardiomyopathy, which involve data for cardiac MRI, ECG, positive family history, and arrhythmia clinics^[71].

Contrast-enhancement-cardiac MRI may help to guide targeted EMBs (Figure 4).

Predilection patterns with midwall contrast enhancement are found in the basal anterior region and/or the RV outflow tract. These patterns of fibrosis correlate with fibrofatty replacement of the myocardium at histological assessment and predict induction of ventricular tachycardia during electrophysiological studies^[69,71].

HYPERTROPHIC CARDIOMYOPATHY

Hypertrophic cardiomyopathy is a clinically heterogeneous autosomal dominant heart muscle disorder with inherited

etiology, primarily by mutations of genes encoding the cardiac sarcomere myofilament proteins. HCM prevalence is 0.2% and one-third of patients show no obstruction of LV outflow tract (LVOT), whereas two-thirds develop a significant gradient under resting conditions and/or on exertion^[72]. HCM was hardly diagnosed in the pre-echocardiographic era and abnormal electrocardiographs suggestive of LVH were attributed by clinicians to hypertensive heart disease. The etiology of HCM has similarly been sorted and HCM is an autosomal dominant genetic disorder, caused by mutations in at least 10 different genes, which code for sarcomeric proteins^[73]. Mutations in the β -myosin heavy chain gene, myosin binding protein C and troponin T account for 70%-80% of all cases. The total number of mutations is > 100 and new mutations are being discovered^[74]. These developments in the etiology of HCM resulted in a change of definition and HCM eventually was no longer a heart muscle disease of unknown cause.

GENETICS IN HCM

Sarcomere mutations are found in 60%-70% of adult and pediatric patients with a family history of HCM and in 30%-40% of apparently sporadic cases^[69]. Mutations in myosin heavy chain (MYH7) and myosin binding protein C (MYBPC3) are the most frequent and comprise up to 8% of cases of sarcomeric HCM. Several studies^[74] have demonstrated that cardiovascular deaths, progressive symptoms, and ventricular arrhythmias appear more prominent in HCM patients with sarcomeric mutations than in patients without mutations. Moreover, patients with more than one mutation have more severe symptomatology^[74]. However the phenotypic presentation and penetrance of mutations may be variable and dependent on several other factors such as presence of hypertension and age. The presence of LVH frequently cannot be diagnosed before adolescence. Thus, the interpretation of genetic testing should be complex including clinical assessment.

The clinical application of genetic testing depends on the confidence of the prediction of disease. Genetic testing must be conducted also as a family test, because its advantages are greatest in larger families with both disease presentations and healthy individuals.

PATHOLOGY IN HCM

HCM is characterized by asymmetrical or symmetrical hypertrophy of the left ventricle with increased LV mass. Asymmetrical hypertrophy is presented by comparing the thickness of the septum with the LV free wall and by presence of septal to free wall thickness ratio > 1.3. Asymmetric hypertrophy of interventricular septum is the most frequent form of HCM. Other presentations include symmetric, apical forms. RV involvement occurs in 17.6% of all cases of HCM, most frequently affecting the middle to apical portion of the right ventricle^[75,76].

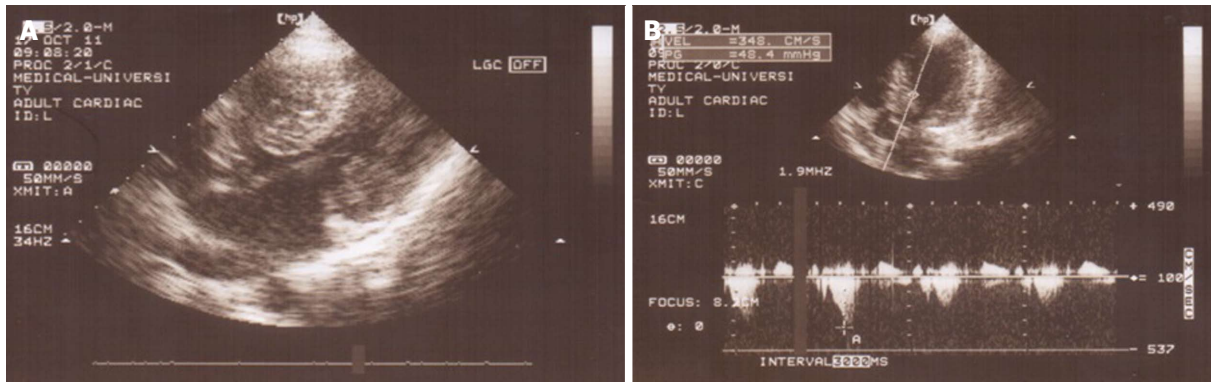


Figure 5 Patient with hypertrophic cardiomyopathy and subaortic stenosis. A: Parasternal long axis view showing expressed left ventricular (LV) hypertrophy at the region of the LV outflow tract; B: Doppler echocardiogram reveals the high subaortic gradient ($\Delta P = 48$ mmHg).

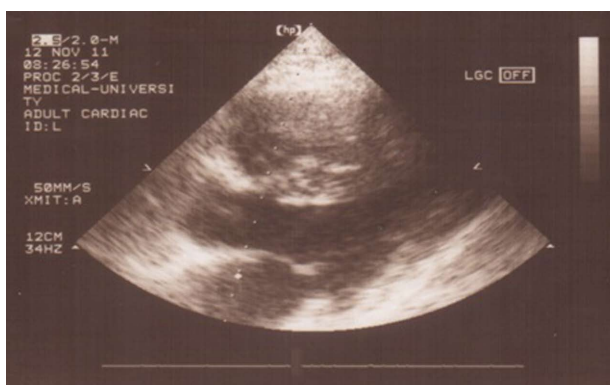


Figure 6 Echocardiogram of a 35-year-old patient with hypertrophic cardiomyopathy: massive hypertrophy of the interventricular septum with wall thickness 37 mm compared to posterior wall; hyperechogenic septal myocardium.

Pathological changes in HCM at the histological level are characterized by cardiomyocyte hypertrophy and disarray with bizarre enlarged nuclei, hyperchromasia and pleomorphism. Increased content of interstitial collagen volume may also be present^[77].

DIAGNOSIS OF HCM

Diagnosis relies on the electrographic and echocardiographic demonstration of hypertrophy patterns. LVH may be diffuse or more segmentally distributed (proximal and/or midportion of the interventricular septum, apex, anterior or lateral wall), but no single morphologic expression appears to be specific^[78].

In fact, differentiation of LVH secondary to HCM may be difficult from other diseases affecting the ventricles, for example, hypertrophy secondary to infiltrative diseases (e.g., amyloidosis), Fabry's disease^[79], glycogen storage disorders^[80], or systemic arterial hypertension. These diagnostic difficulties may rise with advanced age (Figures 5-8).

Besides LVH, LV outflow obstruction is one of the most common features of this disease. Asymmetric basal septal hypertrophy and the systolic anterior motion of the anterior leaflet of the mitral valve are the major con-

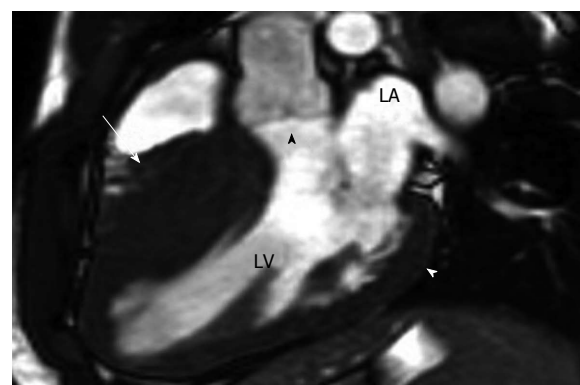


Figure 7 Hypertrophic cardiomyopathy^[105]. A: 2D SSFP cardiac magnetic resonance imaging, obtained in end diastole in the long-axis plane of the LV outflow tract (LVOT) in a 17-year-old boy with Hypertrophic cardiomyopathy found at family screening, shows marked asymmetric septal hypertrophy with a ratio of ventricular septal thickness (27 mm, arrow) to inferolateral wall thickness (9 mm, white arrowhead) of 3:1. Note that the hypertrophied septum encroaches on the LV lumen, causing mild narrowing of the LVOT (black arrowhead). LA: Left atrium; LV: Left ventricle.

tributors of LV outflow obstruction and the more or less significant accompanying mitral regurgitation^[81]. In a series of 320 consecutive HCM patients, this obstructive pathology at resting conditions (defined as a gradient ≥ 50 mmHg at rest) was found in 37% of patients^[82]. In the remaining patients, 52% developed dynamic outflow gradients during exercise or maneuvers which decrease afterload or increase contractility. Abnormal diastolic function is typical pattern of HCM. It may be present at early stages of HCM, even before morphological evidence of hypertrophy occurs^[83,84].

The clinical presentation of HCM patients shows remarkable diversity: some individuals experience none or minor symptoms, others may develop dyspnea at exercise or at rest, angina pectoris, palpitations, atrial fibrillation, dizziness, presyncope and syncope, fatigue or finally end-stage heart failure requiring cardiac transplantation^[85].

The changes on ECG are variable and include left axis deviation, occurrence of Q waves, a positive Sokolow index for hypertrophy, conduction abnormalities, ST-T depression or other abnormalities, negative T waves and giant T waves (particularly observed in Japanese

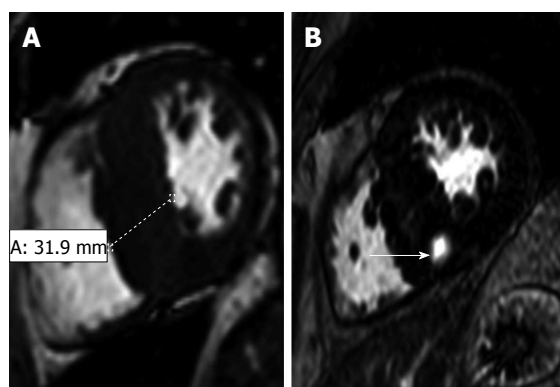


Figure 8 Hypertrophic cardiomyopathy in a 57-year-old man with a 2-year history of exertional dyspnea and chest discomfort who underwent implantable cardioverter-defibrillator placement for primary prevention of sudden cardiac death^[109]. A: Short-axis 2D SSFP magnetic resonance imaging (MRI) performed in end diastole shows asymmetric septal hypertrophy with a maximal thickness of 31.9 mm encroaching on the ventricular lumen; B: Short-axis late contrast-enhanced MRI shows a patchy nodular area of enhancement in the hypertrophied septum (arrow) that does not correspond to a coronary artery territory and, therefore, is distinctly different from an infarct scar.

patients with apical type of HCM^[86]. The ECG abnormalities may not parallel hypertrophy in all cases. Konno *et al*^[73] observed ECG abnormalities (in particular ST-T abnormalities) in about 54% of genetically affected, but nonhypertrophic patients at echocardiography. A normal ECG does not exclude the presence of HCM but suggests a mild manifestation of the disease^[87].

Risk stratification

Identification of high-risk HCM patients is important because of the need to implant an ICD. Several major risk factors of sudden death have been identified to date and these factors are: positive family history of premature SCD caused by HCM, documented nonsustained ventricular tachycardia, syncope at rest or during exercise, abnormal blood pressure response during exercise with increase in the systolic blood pressure of < 20 mmHg from the baseline value, and progressive fall in blood pressure during exercise or a fall in the systolic value by 20 mmHg after an initial increase, particularly in younger patients (< 40 years of age), expressed LVH with wall thicknesses > 30 mm^[88]. The highest rate of cases of SCD in adolescents was linked with pronounced hypertrophy^[88]. Potential additional risk factors include marked fibrosis on cardiac MRI, LV apical aneurysm, LVOT with gradient > 30 mmHg at rest, obstructive sleep apnea^[88].

MANAGEMENT OF HCM

Medical therapy

Many patients with LVOT gradients > 50 mmHg may still be asymptomatic, but most HCM patients have symptoms that need to be managed. β -Blockers represent the cornerstone of therapy and have proved effective in patients with angina or dyspnea on effort, particularly when associated with LVOT obstruction, and are often administered to decrease the frequency of non-

sustained ventricular arrhythmias. These beneficial effects are mediated by negative inotropic, chronotropic effects, improved ventricular relaxation, and increased time for diastolic filling. Despite these advantages, whether long-term treatment with β -blockers ultimately affects outcome in HCM patients remains undefined. By virtue of their efficacy in reducing LVOT obstruction and myocardial ischemia, current guidelines recommend β -blockers as first-line agents in symptomatic patients, both with and without resting obstruction. Two recent studies have consistently shown marked reduction or abolition in exercise-induced LVOT obstruction^[83]. In patients intolerant to β -blockers, verapamil may be a good alternative for treatment of HCM patients. Verapamil and diltiazem have been administered in symptomatic patients with non-obstructive HCM. HCM guidelines suggest caution in using calcium channel blockers in patients with significant LVOT obstruction and elevated pulmonary artery wedge pressure, due to their potentially adverse hemodynamic effects and risk of precipitating edema. The beneficial effects of calcium channel blockers are largely mediated by their negative inotropic and chronotropic effects, leading to prolonged LV filling time and improved redistribution of flow towards the subendocardial layers of the left ventricle. To date, there is no definite evidence that verapamil effectively improves functional capacity in HCM, although the drug has been used for decades to ameliorate quality of life in nonobstructive patients, and is considered standard treatment^[89]. Diltiazem was shown to improve LV diastolic parameters, either acutely or at mid-term administration^[84].

The class IA antiarrhythmic drug disopyramide has been used successfully to attenuate the pressure gradient and improve symptoms in patients with LVOT obstruction, generally in association with β -blockers. The beneficial effect of disopyramide is conditioned by negative inotropic effects, resulting in symptomatic improvement^[90]. Nevertheless, concerns regarding QTc prolongation and significant anticholinergic side effects may limit its long-term use.

Previously it was considered that amiodarone may have a protective role in HCM, with regard to ventricular arrhythmias. However, its efficacy in preventing sudden death is now considered not evident based on the fact that 20% of patients dying suddenly in one retrospective study were on active amiodarone treatment at the time of death^[91].

Several studies showed that approximately two-thirds of patients can be successfully managed by medical therapy with resulting symptoms limitation and decrease of LVOT gradient > 50 ^[89,91,92].

INTERVENTIONAL THERAPY AND SURGERY

Despite advances and efficacy of medical management of patients with HCM, many patients remain symptomatic and at high risk of SCD, which requires interven-

tional approaches to relieve LVOT obstruction. Alcohol septal ablation may be a suitable approach for patients with advanced age and high surgical risks. The procedure involves injecting 1-3 mL 96% ethanol into one of the septal branches supplying the hypertrophied myocardium, causing acute regional contractile dysfunction and leading to a thinning over the long term. This approach leads to reduction or elimination of the obstruction in 90% of cases. Mortality associated with the procedure is similar to that for myectomy (1%-2%) in experienced centers. High-grade AV block as a complication requiring implantation of a pacemaker is registered in experienced centers in 5% of cases^[93].

Septal myectomy using the Morrow procedure has been defined as the therapy standard for many years for patients with HCM, who cannot be adequately treated by pharmacotherapy. The procedure involves removal of a part of the hypertrophied basal septum or thinning of the remaining septum to 5-8 mm. A reduction or elimination of the gradient was achieved in > 90% of patients. The procedure is indicated in patients with symptoms corresponding to NYHA class III and gradient > 50 mmHg (rest or provocation). Perioperative mortality in experienced centers is 1%-2% and the rate of complete AV blocks postoperatively is 2%-5%^[94].

In patients with HCM, pacing the RV apex and apical septum can cause a decrease in the outflow tract gradient by decreasing the ventricular contractility, with a decrease in systolic movement of the basal septum to the LVOT. Continuous pacing with the development of LV enlargement may further decrease LVOT gradient. Dual chamber pacing has shown modest benefit in randomized controlled trials. It is mostly indicated in patients > 65 years of age, those who have indication for pacemaker or ICD implantation, and those who have a high risk of surgery^[95].

RESTRICTIVE CARDIOMYOPATHIES

Restrictive cardiomyopathy is a disease of the myocardium characterized by impaired ventricular filling and reduced diastolic volume of either or both ventricles, with normal or near-normal systolic function.

Unlike DCM and HCM, where the definition is morphological, the definition of restrictive cardiomyopathy is based on hemodynamic abnormalities. Myocardial relaxation abnormality with interstitial fibrosis and calcifications compose the fundamental abnormalities of restrictive cardiomyopathies. Restrictive filling is due to higher diastolic pressure and causes passive venous congestion. Cardiac output can be increased by an increase of heart rate, but becomes ineffective due to shortened filling time.

PREVALENCE

Restrictive cardiomyopathies form 5% of pediatric cardiomyopathies, but several types are more common in

certain populations. For example, EMF is a relatively common cause of heart failure in equatorial Africa^[96].

PATHOPHYSIOLOGY AND CLINICAL MANIFESTATIONS

These conditions result in impaired ventricular filling and primarily diastolic heart failure. They manifest with a clinical heart failure syndrome frequently indistinguishable from that caused by systolic dysfunction. AV block and symptomatic bradycardia can be seen, often indicating pacemaker insertion. Atrial fibrillation is poorly managed by conventional therapy.

Restrictive cardiomyopathies may be classified as primary (*e.g.*, EMF, Löffler's endocarditis, and idiopathic restrictive cardiomyopathy) or secondary. Causes of secondary restrictive cardiomyopathy include infiltrative diseases (*e.g.*, amyloidosis, sarcoidosis, and radiation carditis) and storage diseases (*e.g.*, hemochromatosis, glycogen storage disorders, and Fabry's disease). Fabry's disease, although rare, has assumed a new importance as effective therapy became possible.

Physical examination in restrictive cardiomyopathies may reveal congestive heart failure signs: peripheral edema, jugular vein distensions, and gallop rhythm. Echocardiographic typical signs of restrictive cardiomyopathy are normal ventricular dimensions with dilated atria as a feature of systemic venous congestion, normal or nearly normal systolic function. Myocardial calcifications are typical for EMF. Some patterns revealed by echocardiography may indicate etiology like granular sparkling of myocardium in amyloidosis (Figure 9), endocardial thickening and thrombus in eosinophilic endocardial disease and EMF.

Doppler features of restrictive cardiomyopathy are high early filling E/A wave ratio > 2, short isovolumic relaxation time < 60 ms, short deceleration time < 150 ms, and expressed pulmonary ravenous reversal flow^[97]. The treatment of restrictive cardiomyopathy patients is mainly symptomatic with diuretics and aldosterone antagonists. Severity of heart failure symptoms and absence of efficacy are the indications for cardiac transplantation^[98].

SPECIFIC TYPES OF RESTRICTIVE CARDIOMYOPATHIES

Amyloidosis

Amyloid heart disease is classified as primary, secondary, familial, or senile. Primary amyloid heart disease is caused by overproduction of amyloid light chain immunoglobulin from a monoclonal population of plasma cells, usually associated with multiple myeloma. Secondary amyloid heart disease is associated with chronic inflammatory conditions such as rheumatoid arthritis, tuberculosis, and familial Mediterranean fever^[99,100].

Familial and senile amyloid heart disease is related to the overproduction of transthyretin. Myocardial amyloid



Figure 9 Patient with secondary cardiac amyloidosis due to familial Mediterranean fever. Echocardiogram shows hypertrophic amyloid infiltration and increased hyperechoic “granular sparkling” myocardium with increased myocardial wall thickness.

heart disease is confirmed by EMB (Figure 10). The presence of near-normal LV dimensions combined with increased myocardial wall thickness, particularly biventricular thickening, should arouse suspicion of an infiltrative cardiomyopathy, especially if accompanied by low-voltage QRS complexes on ECG. Unfortunately, there is no proven treatment for cardiac amyloidosis and the prognosis remains poor.

HEMOCHROMATOSIS

Hemochromatosis (“bronze diabetes”) is a disease that results in iron overload and deposition of iron in the sarcoplasmic reticulum of many organs, including the heart. Most commonly it has autosomal recessive type of Mendelian inheritance. Typically, this disorder has multi-system manifestations. Erythropoiesis remains normal, but progressive parenchymal iron deposition causes multi-organ insufficiencies. Excess of cellular iron leads to cellular death and fibrosis^[101]. The use of serum ferritin levels as a screen for this condition may be clinically important. Cardiac MRI can have diagnostic value to reveal cardiac involvement. Hemochromatosis may result in a restrictive or dilated cardiomyopathy, with characteristic histological features. Treatment is by repeated phlebotomy. Family screening is advised.

SARCOIDOSIS

Sarcoidosis is a systemic disease resulting in the formation of noncaseating granulomas that can infiltrate the myocardium. It is associated with restrictive cardiomyopathy in 5% of patients, but may later progress to DCM^[102]. It is difficult to diagnose unless there is other organ involvement (usually pulmonary). It may be suspected in patients with cardiomyopathy and lymphadenopathy, skin rashes, or splenomegaly. Cardiac sarcoidosis is associated with ventricular tachycardia and conduction abnormalities (especially complete heart block) that can cause syncope and SCD. EMB may show findings specific for sarcoidosis but, because of the patchy nature of the disease, biopsy

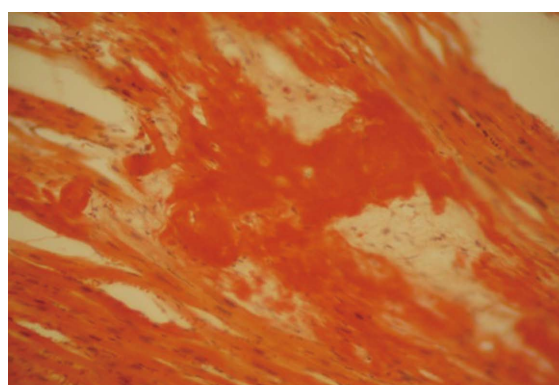


Figure 10 Amyloid deposits in myocardium in patient with secondary amyloidosis due to familial Mediterranean fever. Autopsy study with Congo-Red-positive extracellular deposits, causing disorder of myocardial organization.

may miss characteristic lesions, resulting in a low overall sensitivity. Cardiac granulomas may occasionally respond to steroids but turn to scar tissue^[103]. Sudden death cannot be prevented by steroids^[104]. Regular Holter monitoring is recommended to look for AV blocks, which should be treated with permanent pacemakers.

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Pulmonary arterial hypertension related to human immunodeficiency virus infection: A case series

Inês Araújo, Cristina Enjuanes-Grau, Carmen Jimenez Lopez-Guarch, Dariusz Narankiewicz, Maria J Ruiz-Cano, Teresa Velazquez-Martin, Juan Delgado, Pilar Escribano

Inês Araújo, 3rd Medicine Department, São Francisco Xavier Hospital, CHLO, 1449-005 Lisboa, Portugal

Cristina Enjuanes-Grau, Cardiology Department, University Hospital of Canarias, 38320 La Laguna, Tenerife, Spain

Carmen Jimenez Lopez-Guarch, Maria J Ruiz-Cano, Teresa Velazquez-Martin, Juan Delgado, Pilar Escribano, Pulmonary Hypertension Unit, University Hospital of 12 de Octubre, 28041 Madrid, Spain

Dariusz Narankiewicz, Internal Medicine Department, Carlos Haya Hospital, 29010 Málaga, Spain

Author contributions: Araújo I, Enjuanes-Grau C, Ruiz-Cano MJ and Escribano P designed the research; Araújo I, Enjuanes-Grau C and Narankiewicz D performed the research; Lopez-Guarch CJ and Velazquez-Martin T contributed diagnostic tools; Araújo I, Enjuanes-Grau C, Ruiz-Cano MJ and Escribano P analysed the data; Araújo I and Escribano P wrote the paper; and Delgado J and Escribano P revised the manuscript.

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Correspondence to: Inês Araújo, MD, 3rd Medicine Department, São Francisco Xavier Hospital, CHLO, Estrada do Forte do Alto do Duque, 1449-005 Lisboa, Portugal. inesarauj@gmail.com

Telephone: +351-21-0431104 Fax: +351-21-0431093

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Abstract

AIM: To present 18 new cases of human immunodeficiency virus (HIV)-related pulmonary arterial hypertension (PAH) with presenting features, treatment options and follow-up data.

METHODS: This is a single-centre, retrospective, observational study that used prospectively collected data, conducted during a 14-year period on HIV-related PAH patients who were referred to a pulmonary hy-

pertension unit. All patients infected with HIV were consecutively admitted for an initial evaluation of PAH during the study period and included in our study. Right heart catheterisation was used for the diagnosis of PAH. Specific PAH treatment was started according to the physician's judgment and the recommendations for idiopathic PAH. The data collected included demographic characteristics, parameters related to both HIV infection and PAH and disease follow-up.

RESULTS: Eighteen patients were included. Intravenous drug use was the major risk factor for HIV infection. Risk factors for PAH, other than HIV infection, were present in 55.5% patients. The elapsed time between HIV infection and PAH diagnoses was 12.2 ± 6.9 years. At PAH diagnosis, 94.1% patients had a CD4 cell count > 200 cells/ μ L. Highly active antiretroviral therapy (present in 47.1% patients) was associated with an accelerated onset of PAH. Survival rates were 93.8%, 92.9% and 85.7% at one, two and three years, respectively. Concerning specific therapy, 33.3% of the patients were started on a prostacyclin analogue, and the rest were on oral drugs, mainly phosphodiesterase-5 inhibitors. During the follow-up period, specific therapy was de-escalated to oral drugs in all of the living patients.

CONCLUSION: The survival rates of HIV-related PAH patients were higher, most likely due to new aggressive specific therapy. The majority of patients were on oral specific therapy and clinically stable. Moreover, sildenafil appears to be a safe therapy for less severe HIV-related PAH.

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Key words: Human immunodeficiency virus infection; Pulmonary arterial hypertension; Treatment

Core tip: Human immunodeficiency virus (HIV)-related

pulmonary arterial hypertension (PAH) is a rare disease, and HIV-infected patients are seldom included in clinical trials. Therefore, case reports are crucial to better understand this disease and its response to specific therapies. In this retrospective, observational study, 18 HIV-related PAH patients were included. Highly active antiretroviral therapy was associated with an accelerated onset of PAH. The survival rates of HIV-related PAH patients were higher, most likely due to new aggressive specific therapy. The majority of patients were on specific oral therapy and were clinically stable. Furthermore, sildenafil appears to be a safe option for less severe disease.

Araújo I, Enjuanes-Grau C, Lopez-Guarch CJ, Narankiewicz D, Ruiz-Cano MJ, Velazquez-Martin T, Delgado J, Escibano P. Pulmonary arterial hypertension related to human immunodeficiency virus infection: A case series. *World J Cardiol* 2014; 6(6): 495-501 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i6/495.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i6.495>

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a progressive disease that is caused by the chronic obstruction of small pulmonary arteries, leading to right ventricular failure and potential death^[1]. Idiopathic and inherited forms have been described. However, this condition is associated with connective tissue diseases, portal hypertension, congenital heart disease, drugs, toxins and human immunodeficiency virus (HIV) infection^[2].

Before the introduction of highly active antiretroviral therapy (HAART), HIV-related PAH was underdiagnosed due to the patients' short survival, which was primarily caused by opportunistic infections. After the introduction of this novel antiretroviral therapy scheme, long-term cardiovascular complications, such as PAH, have emerged^[3].

The first case of HIV-related PAH was described in 1987 in an HIV-infected subject with haemophilia and membranoproliferative glomerulonephritis^[4]. Subsequently, several other cases have been reported. Nonetheless, HIV-related PAH is a rare disease: in 1991, prior to the introduction of HAART, the prevalence of HIV-related PAH was estimated to be 0.5% in developed countries^[5]. This rate is 25-fold higher than the prevalence of PAH in the general population^[6].

Recent studies have shown that prevalence has not changed in recent years. As described by Sitbon *et al*^[7], the prevalence is 0.46%, suggesting that HAART does not prevent HIV-related PAH. However, because most published studies do not include asymptomatic patients, the actual prevalence could be higher. In 2008, Reinsch *et al*^[8] found that the prevalence in asymptomatic patients is 4.8%, although the diagnosis of PAH was only based on echocardiographic parameters. HIV-related PAH is clinically

and histologically similar to idiopathic PAH^[3].

The aim of this study is to present 18 new cases of HIV-related PAH with presenting features, treatment options and follow-up data.

MATERIALS AND METHODS

This is a single-centre, retrospective, observational study using prospectively collected data that was conducted over a 14-year period between June 1998 and June 2012.

All HIV-infected patients consecutively admitted to the Pulmonary Hypertension Unit of Hospital 12 De Octubre for an initial evaluation of PAH during the study period were included in our study.

PAH was diagnosed with right heart catheterisation and defined by a resting mean pulmonary arterial pressure of more than 25 mmHg and a pulmonary capillary wedge pressure of less than 15 mmHg. Poor prognostic factors included a right atrium pressure (RAP) > 15 mmHg and a cardiac output ≤ 2.0 mL/min^[2,9].

No specific recommendations for the treatment of PAH-HIV have been made thus far; therefore, specific PAH treatment was initiated according to the physician's judgment and the recommendations for idiopathic PAH treatment. HAART is a combination of at least 3 antiretroviral drugs, such as 3 nucleoside reverse transcriptase inhibitors, 2 nucleoside reverse transcriptase inhibitors and 1 protease inhibitor or 2 nucleoside reverse transcriptase inhibitors and 1 non-nucleoside reverse transcriptase inhibitor.

All patients received nonspecific supportive therapy as recommended, unless such therapy was contraindicated or not necessary: oral anticoagulation to maintain an international normalised ratio of 2.0-3.0, long-term oxygen therapy if there was evidence of hypoxemia and diuretic therapy for right heart failure symptomatic control.

Baseline evaluation included an assessment of the NYHA functional class, 6-Minute Walk Distance (6MWD) and echocardiogram. A re-evaluation of right heart catheterisation was only performed if clinical worsening occurred. Follow-up was conducted indefinitely for alive patients or until death.

The data collected included demographic characteristics (age, gender), parameters related to both HIV infection and PAH and disease follow-up. The date of PAH diagnosis was used as the baseline for survival estimates.

Because patients had already consented to be included in the PAH national registry, no additional informed consent was needed for this sub-study.

Statistical analysis

Standard descriptive statistics were used. Variables such as New York heart association (NYHA) functional class and 6MWD were compared using the paired-sample *t*-test. Due to the small population size, univariate analysis was not possible.

All statistical tests were performed using SPSS for Windows (version 16.0: SPSS, Chicago, IL, United States).

Table 1 Patients' demographic and clinical characteristics at pulmonary hypertension diagnosis

Case No.	Age, yr	Gender	HIV risk factor	Duration of HIV infection previous to PAH, yr	CD4 count, cells/ μ L	HIV viral load, copies/mL	CDC stage	HAART	History of viral hepatitis	Other PAH risk factors	NYHA FC
1	25	F	IVDU	12	339	< 50	B3	No	Hep C	None	IV
2	39	F	IVDU	4	700	< 50	A1	No	Hep B + C	None	II
3	39	M	IVDU	11	460	< 50	B3	Yes	No	ASD	III
4	32	M	IVDU	12	500	< 50	B2	No	Hep C	ASD	III
5	43	M	UK	7	397	< 50	B3	Yes	No	Splenectomy without CTEPH	III
6	45	M	IVDU	8	332	< 50	B3	Yes	Hep B+C	Portal Hypertension	II
7	40	F	IVDU	22	517	< 50	C3	UK	Hep C	None	II
8	31	F	Hetero-sexual	1	444	< 50	A2	Yes	No	None	III
9	40	M	IVDU	22	900	3000	A1	No	Hep C	None	II
10	41	M	IVDU	21	772	308	A2	No	Hep C	None	II
11	40	M	IVDU	10	460	359810	A1	Yes	Hep C	Portal Hypertension	III
12	40	F	UK	5	46	114	C3	No	Hep C	Portal Hypertension	III
13	39	M	IVDU	10	1234	< 50	B1	Yes	Hep C	None	III
14	46	M	IVDU	14	411	< 50	A2	Yes	Hep C	Portal Hypertension	II
15	47	F	IVDU	14	800	< 50	A1	No	Hep C	ASD	III
16	47	M	IVDU	4	450	< 20	A2	Yes	Hep C	None	II
17	47	F	IVDU	23	760	< 20	C2	No	Hep B + C	Portal Hypertension	II
18	43	M	UK	19	NA	NA	A2	No	No	Portal Hypertension	III

ASD: Atrial septal defect; CDC: Centers for disease control; CTEPH: Chronic thromboembolic pulmonary hypertension; F: Female; M: Male; HAART: Highly active anti-retroviral therapy; HIV: Human immunodeficiency virus; IVDU: Intravenous drug use; NA: Not accessible; NYHA FC: New York heart association functional class; PAH: Pulmonary arterial hypertension; UK: Unknown.

RESULTS

Eighteen patients were admitted to our centre during the study period, and their baseline characteristics are listed in Table 1. Male gender was predominant (61.1%), and the mean age was 40.2 years (range 25-47 years).

Concerning HIV infection, intravenous drug use was the major risk factor for infection (77.8%). At PAH diagnosis, only 16.7% were in Centers for Disease Control and Prevention stage C. The mean CD4 cell count was 554 ± 267 cells/ μ L, with only one patient having a CD4 cell count < 200 cells/ μ L. The viral load was undetectable in 76.5% of the patients.

The mean time interval between HIV infection diagnosis and PAH diagnosis was 12.2 ± 6.9 years (range 1-23 years). Approximately half of the patients were on HAART; among those who were not on HAART, only three patients had an indication for HIV treatment before the diagnosis of PAH (one was not under treatment at all due to poor adherence, and the other two took only two drugs: a non-nucleoside reverse transcriptase inhibitor and a protease inhibitor). There is a statistically significant acceleration in the onset of PAH in patients on HAART ($P = 0.012$).

Concomitant viral hepatitis (either C or C and B) was present in 77.8% of the patients. In 55.5% of the patients, other risk factors for PAH were identified (Table 1), including portopulmonary hypertension, splenec-

tomy and congenital heart defects. An atrial septal defect (ASD) was present in three patients: two patients had the ostium secundum type with a bidirectional shunt and a diameter of 1.8 and 0.7 cm, and the other patient had Eisenmenger's syndrome due to a sinus venosus type ASD with a 3.0 cm diameter. At PAH diagnosis, 55.6% of the patients were in the NYHA functional classes III-IV. Shortness of breath was the most prevalent symptom (present in all patients), followed by chest pain (27.8%), syncope (22.2%) and peripheral oedema and ascites (5.6%).

Exercise capacity was assessed by the 6MWD (Table 2), with a mean achieved distance of 436 ± 113 m. All but one patient walked 300 m or more at diagnosis. There was an overall improvement in the test, but no significant difference was found among the patients with or without HAART ($P = 0.401$).

Hemodynamic parameters, as assessed by right heart catheterisation, showed a mean pulmonary artery pressure (mPAP) of 52.6 ± 12.2 mmHg (17.6% of patients had a mPAP between 35-40 mmHg, 23.5% were between 41-45 mmHg and 58.8% were > 45 mmHg), a RAP of 6.1 ± 3.8 mmHg (none of the patients were > 15 mmHg) and a cardiac output of 4.6 ± 1.4 mL/min (11.7% of the patients with a cardiac output ≤ 2.0 mL/min).

The mean follow-up period was 5.8 ± 4.2 years, with a minimum of 0.3 and a maximum of 11.8 years (Table 2). Overall, the patients had an improved NYHA functional class, with a reduction of approximately half a functional

Table 2 Patients' follow-up data

Case No.	Actual status	Period of follow-up, yr	Year of PAH diagnosis	Initial specific therapy	Last visit specific therapy	Initial 6MWT (m)	Final 6MWT (m)	Variation on NYHA FC
1	Dead (cause: heart failure)	2.8	1998	Epoprostenol	Epoprostenol	211	463	0
2	Alive	11.3	2001	Treprostinil	Sildenafil	NA	669	-1
3	Alive	10.8	2001	Sildenafil	Sildenafil + Ambrisentan	512	630	-1
4	Alive	9.9	2002	Sildenafil	Sildenafil + Ambrisentan + Iloprost	516	570	-2
5	Alive	11.3	2001	Treprostinil	Sildenafil	313	414	0
6	Dead (cause: liver disease)	0.3	2005	Sildenafil	Sildenafil	400	NA	NA
7	Alive	7.0	2005	Ambrisentan	Ambrisentan + Sildenafil	435	473	0
8	Missing	3.9	2006	Sildenafil	Sildenafil	300	511	-2
9	Alive	5.3	2006	Sildenafil	Sildenafil	455	546	0
10	Alive	4.5	2007	Sildenafil	Tadalafil	650	703	0
11	Alive	8.5	2003	Treprostinil	Tadalafil	510	570	-2
12	Alive	8.8	2003	Iloprost	Ambrisentan	NA	450	-1
13	Alive	11.8	2000	Treprostinil	Bosentan	327	489	-1
14	Missing	0.4	2005	Sildenafil	Sildenafil	500	NA	0
15	Alive	3.5	2008	Sildenafil + Bosentan	Sildenafil + Bosentan + Iloprost	350	420	0
16	Alive	1.6	2010	Tadalafil	Tadalafil	525	570	0
17	Alive	1.4	2011	Ambrisentan	Ambrisentan	439	423	0
18	Alive	0.5	2011	Sildenafil	Sildenafil	537	600	0

6MWT: 6 min walk test; NYHA FC: New York heart association functional class; NA: Not accessible; PAH: Pulmonary arterial hypertension.

class per patient ($P = 0.008$) and an improvement of 85 m on the 6MWD ($P = 0.002$). Functional class improvement was not dependent on HAART therapy ($P = 0.343$).

At PAH diagnosis, six (33.3%) patients were started on prostacyclin analogues. One patient was started on epoprostenol but died due to right heart failure. Recurrent infections of the Hickman catheter, which the patient used for drug abuse, might have played a role. At the last registered visit, the other five patients had clinically improved and reduced the specific therapy to oral drugs, such as phosphodiesterase-5 inhibitors and endothelin receptor antagonist (Table 2).

All but one of the remaining patients were started on specific oral monotherapy. On their most recent follow-up, these patients were on the same therapy, except for four patients who needed other specific drugs (two were started on dual combined therapy, and the other two were started on triple combined therapy). An improvement in NYHA functional class and 6MWD was observed in half and all of these patients, respectively. Specific oral therapy was well tolerated in all patients, without any major documented adverse reactions, except elevated liver enzymes in one patient on sildenafil, in whom the specific therapy was changed to tadalafil.

Concerning survival during the follow-up period, two patients died (11.1%), but only one death was related to PAH (right heart failure); this patient had one of the lowest CD4 cell counts (339 cells/ μ L). The other, as already discussed, had the worst NYHA functional class at PAH diagnosis; this death was related to chronic liver disease, and the patient died soon after PAH diagnosis. Two patients were lost to follow-up (Table 2). At 1, 2 and

3 years, the survival rates were 93.8%, 92.9% and 85.7%, respectively.

DISCUSSION

This study reports data on 18 HIV-infected patients diagnosed with PAH. HIV-infected patients are at a higher risk of developing PAH compared with the general population. Nevertheless, the global prevalence of this disease is low, and HIV-infected patients are rarely included in clinical trials because of the risk of interaction between PAH therapies and anti-retrovirals and the presence of multiple comorbidities in HIV-infected patients. Therefore, case reports and case series have become crucial to determine the characteristics of the disease and the efficacy of therapy. Despite our small number of patients, a major contribution with regard to survival rates and specific therapy can be made. In our study group, male gender was predominant, which is concordant with other authors and may reflect the high prevalence of men in the HIV population^[10-12]. However, in a recent cross-sectional study of 802 HIV-infected patients, 14 patients had symptomatic PAH, and women were more affected, with a male: female ratio of 1:1.4^[8]. Age at diagnosis did not differ from other studies that have reported a mean age ranging from 32 to 43 years^[6,10-14].

Intravenous drug use was the most prevalent risk factor among HIV-infected patients with PAH (77.8% of the patients). This high prevalence was also described in other studies^[10,12,13,15]. Nonetheless, patients with PAH related to HIV infection acquired *via* intravenous drug abuse have no clinical, functional or hemodynamic speci-

ficiencies, compared with patients with PAH related to HIV infection from any other route of transmission^[15].

The mean CD4 count at the time of PAH diagnosis was somewhat higher than those observed in other studies, with only one patient (5.8%) having a CD4 cell count < 200 cells/ μ L; the viral load was undetectable in the majority of patients. A CD4 cell count < 200 cells/ μ L was observed in 59.6% and 52% of patients in the studies of Zuber *et al.*^[12] and Nunes *et al.*^[15], respectively. This difference may reflect the efficacy of the actual antiretroviral therapy. Hence, HIV-related PAH occurs in the early and late stages of HIV infection and may not be related to viral load or immune status, partially demonstrating that HAART does not prevent PAH^[6,8,15].

The median time interval between the diagnoses of HIV disease and PAH, as described in literature, has ranged from 2.8 to 7.7 years^[6,10-12,15], which is much shorter than the time interval determined in our study (11.5 years). Degano *et al.*^[13] also described a more prolonged time interval (11 years), suggesting that HAART does not prevent but may delay the development of PAH in HIV-infected patients. However, in our study, HAART was found to accelerate the onset of PAH, and this finding has been corroborated by Reinsch *et al.*^[8] and Pellicelli *et al.*^[16]. This may be due to a closer monitoring of patients on HAART, enabling an earlier PAH diagnosis.

In our population, 55.5% of the patients had another PAH risk factor other than HIV infection, including atrial septal defect, splenectomy and portal hypertension, which differs from the results of other papers. In the study by Humbert *et al.*^[17], approximately 4% of PAH patients presented with two co-existing risk factors, mainly HIV infection with portal hypertension. Mesa *et al.*^[18] reported that 13% of the HIV-related PAH patients had coexistent liver disease. Other causes of PAH and chronic thromboembolic pulmonary hypertension were not reported in our patients and have been rarely reported in HIV-infected patients^[19].

The majority of the patients in our study were in a high NYHA functional class, which is consistent with other authors who have reported 71%-81% of patients in the NYHA classes III-IV at diagnosis^[15,17].

The survival rates were far better than those described in the literature. During the follow-up period of 5.8 ± 4.2 years, 12.5% patients died, and the survival rates at one, two and three years was 93.8%, 92.9% and 85.7%, respectively. In the study by Degano *et al.*^[13], the survival rates were closely related to our findings: 88%, 84%, 72% and 63% at one, two, three and five years, respectively, but others have reported lower survival rates^[14,15,20]. Mortality in patients with HIV-related PAH is usually due to right heart failure, rather than other complications of HIV infection, and PAH is considered an independent predictor of death in HIV-infected patients^[10,15]. This finding may relate to the fact that most of these individuals present in the later stages of PAH. In our study, the patient who died due to right heart failure had the worst NYHA functional class and showed no functional or hemodynamic improvement despite ag-

gressive therapy.

Apart from the effect on PAH development, HAART has been described to influence prognosis. In our population, the improvement in NYHA functional class did not differ between the groups, nor did exercise capacity, as measured by 6MWD. Concerning mortality, the patient who died due to right heart failure was not on HAART; the other patient who died was on HAART, but the cause of death was liver failure. HAART has been associated with an improvement in exercise capacity, NYHA functional class, right ventricular systolic pressure over right atrial pressure gradient and overall survival^[11-14,21,22]. However, there is also contradictory data stating that patients without HAART have no reduction in survival^[23]. No current trial has been designed to evaluate the effect of HAART on the progression of HIV-PAH, but due to the weight of scientific information favouring HAART, patients with HIV-related PAH should be treated with HAART, irrespective of their CD4 cell counts^[14]. This suggestion is also supported by our study.

An analysis of the cases of HIV-related PAH reported in the literature (from January 1987 to January 2009) showed a better outcome in patients treated with PAH-specific therapy than in those treated with just antiretroviral therapy^[24]. Regardless of the substantial progress in therapy over the last few years, no randomised study has established a drug of choice for the treatment of HIV-related PAH. The evidence for the use of bosentan and prostaglandin therapies comes from cohort studies, case-control studies or case series. Therefore, the treatment of HIV-related PAH relies on PAH-specific therapy and includes supportive treatments and disease-specific treatments.

In our population, six of the 18 patients were started on prostacyclin analogues. These were patients with worse functional status who were diagnosed between 1998 and 2003, when prostacyclin analogues were the only recommended specific therapy for PAH. Only one of these patients was on epoprostenol due to advanced disease. The other patients were started on either iloprost or treprostinil. The beneficial effects of this drug class have been demonstrated in patients with HIV-related PAH. Our patients on iloprost and treprostinil have shown an improvement in NYHA functional class, and treatment was de-escalated to oral drugs. De-escalation to specific oral therapy can be attempted in stable patients with good long-term progress. Favourable results have been noted when switching from prostacyclin and its analogues to bosentan, with the clinical stability and pulmonary pressure measurements being maintained^[25]. Similarly, transitioning from subcutaneous treprostinil to sildenafil was safely demonstrated in patients with PAH of varied aetiologies^[26].

Sildenafil was the most commonly used drug among our patients (61.1%), though tadalafil was also used (16.6%). In the majority of patients, the clinical results were satisfactory. However, the experience with phosphodiesterase-5 inhibitors in HIV-related PAH is preliminary, and no controlled studies exist. Beneficial effects derived

from case studies have been reported, including improvements in dyspnoea, NYHA functional class, exercise capacity and mPAP^[27-29]. However, because sildenafil is largely metabolised by cytochrome P450 3A4, there is a potential for drug interactions when it is co-administered with several antiretroviral therapies, particularly protease inhibitors. Therefore, sildenafil is rarely used. In a review of 154 case reports by Janda *et al.*^[11], phosphodiesterase-5 inhibitors were the least commonly used therapy and were only an option for patients who did not tolerate bosentan. Our study reinforces the benefits of sildenafil HIV-related PAH therapy, and few adverse events were reported.

This study has some limitations. This is a retrospective study conducted in a single centre, with possible biases. However, it would be ethically impossible to perform a prospective study designed to compare novel therapies such as prostacyclin analogues with less active treatments in a cohort of patients with HIV-related PAH. Because HIV-related PAH is a rare disease, few patients were included in this study, thus any analyses should be considered cautiously.

To summarise, this study adds important information to what has been reported in the literature. Survival rates of HIV-related PAH patients tend to be higher, which may be due to new aggressive specific therapies, such as prostanoids, but not to HAART. The majority of the patients were treated with specific oral therapy, even those primarily treated with prostacyclin analogues.

The onset and rapid progression of shortness of breath and other cardiopulmonary symptoms in HIV-infected individuals should suggest HIV-related PAH. A systematic cardiopulmonary evaluation and follow-up in specialised centres should be incorporated into the clinical management of HIV-infected patients to enhance quality of life, exercise capacity and survival through the delivery of HAART and specific therapy.

COMMENTS

Background

Pulmonary arterial hypertension (PAH) is a progressive disease that is caused by the chronic obstruction of small pulmonary arteries, leading to right ventricular failure and potential death. Idiopathic and inherited forms have been described.

Research frontiers

After the introduction of this novel antiretroviral therapy scheme, long-term cardiovascular complications, such as PAH, have emerged. The first case of human immunodeficiency virus (HIV)-related PAH was described in 1987 in an HIV-infected subject with haemophilia and membranoproliferative glomerulonephritis. Subsequently, several other cases have been reported. Nonetheless, HIV-related PAH is a rare disease: in 1991, prior to the introduction of highly active antiretroviral therapy (HAART), the prevalence of HIV-related PAH was estimated to be 0.5% in developed countries. This rate is 25-fold higher than the prevalence of PAH in the general population.

Innovations and breakthroughs

Recent studies have shown that prevalence has not changed in recent years. This study was designed to present 18 new cases of HIV-related PAH with presenting features, treatment options and follow-up data.

Applications

In this series, the survival rates of HIV-related PAH patients tended to be higher,

but this difference was not related to HAART. The majority of patients were treated with specific oral therapy, even in those who were primarily treated with prostacyclin analogues. The onset and rapid progression of shortness of breath and other cardiopulmonary symptoms in HIV-infected individuals can suggest HIV-related PAH, and its prompt identification and treatment can improve quality of life, exercise capacity and survival.

Term explanation

HAART: the use of multiple drugs that act on different viral targets to decrease the patient's total burden of HIV, to maintain the immune system's function and to prevent opportunistic infections. Prostacyclin analogues (epoprostenol, iloprost and treprostinil): drugs used in pulmonary arterial hypertension that reduce pulmonary pressure and improve right ventricular stroke work.

Peer review

This is an excellent review of 18 cases about PAH related to HIV infection. The authors have stated the differences with previous studies, including the time interval between the HIV and PAH diagnoses, PAH risk factors other than HIV infection, HAART, specific therapy and survival rates.

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Respiratory modulation of cardiac vagal tone in Lyme disease

Basant K Puri, Mussadiq Shah, Jean A Monro, Michele C Kingston, Peter OO Julu

Basant K Puri, Department of Medicine, Imperial College London, Hammersmith Hospital, London W12 0HS, United Kingdom
Basant K Puri, Imaging Directorate, Imperial College London, Hammersmith Hospital, London W12 0HS, United Kingdom
Mussadiq Shah, Jean A Monro, Michele C Kingston, Peter OO Julu, Department of Neuroscience, Breakspear Medical Group, Hertfordshire House, Hemel Hempstead, Hertfordshire HP2 4FD, United Kingdom

Peter OO Julu, Department of Medicine, Imperial College London, London SW7 2AZ, United Kingdom

Peter OO Julu, National Rett Center, SE-832 23 Frösön, Sweden
Author contributions: Puri BK designed the study, analyzed the data, and drafted the article; Shah M, Monro JA, Kingston MC and Julu POO contributed to the conception of the study, acquired the data, and critically reviewed the article.

Correspondence to: Basant K Puri, MA, PhD, MB, BChir, BSc (Hons) MathSci, DipStat, MMath, FRCPsych, FSB, Professor, Imaging Directorate, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0HS, United Kingdom. basant.puri@imperial.ac.uk

Telephone: +44-1442-261333 Fax: +44-1442-266388

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CONCLUSION: Respiratory modulation of cardiac vagal tone is impaired in Lyme disease, which suggests that Lyme disease may directly affect the vagus nerve or the brainstem.

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Key words: Cardiac vagal tone; Lyme disease

Core tip: Given that immune dysfunction, postural orthostatic tachycardia syndrome, fatigue, cognitive dysfunction, orthostatic palpitations, syncope, and stress, which may occur in Lyme disease, are associated with parasympathetic activity and reduced modulation of cardiac vagal tone, we hypothesized that modulation of cardiac vagal tone is impaired in this disease. This was confirmed in our study of 18 patients and 18 matched controls. Cardiac vagal tone is reflexly generated through arterial baroreceptor stimulation, by the afferents of the latter facilitating cardiac vagal motoneuron discharge relaying through interneurons in the nucleus tractus solitarius, implying that Lyme disease may directly affect the vagus or brainstem.

Abstract

AIM: To conduct the first systematic test of the hypothesis that modulation of cardiac vagal tone is impaired in Lyme disease.

METHODS: The response of cardiac vagal tone to respiratory modulation was measured in 18 serologically positive Lyme disease patients and in 18 controls.

RESULTS: The two groups were matched in respect of age, sex, body mass, mean arterial blood pressure, mean resting heart rate and mean resting cardiac vagal tone. The mean maximum cardiac vagal tone during deep breathing in the Lyme disease patients [11.2 (standard error 1.3)] was lower than in the matched controls [16.5 (standard error 1.7); $P = 0.02$].

Puri BK, Shah M, Monro JA, Kingston MC, Julu POO. Respiratory modulation of cardiac vagal tone in Lyme disease. *World J Cardiol* 2014; 6(6): 502-506 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i6/502.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i6.502>

INTRODUCTION

Lyme disease or Lyme borreliosis is an arthropod-borne zoonosis caused by *Borrelia* spirochetes, the incidence of which has recently been increasing with the geographical spread of infected ticks and which was previously identified clinically in Europe as Garin-Bujadoux-Bannwarth syndrome and in the United States as Lyme arthritis^[1-3]. There is growing evidence for the role of the autonomic

nervous system in a wide range of diseases^[4] and autonomic instability has been reported in Lyme disease^[5] but has not, thus far, been systematically studied in this illness. It has recently been reported that a series of five female Lyme disease patients developed postural orthostatic tachycardia syndrome; they suffered from symptoms of fatigue, cognitive dysfunction, orthostatic palpitations and either near syncope or frank syncope^[6]. Again, a case report has been published of a 16-year-old female patient with clinical, radiological and scintigraphic features consistent with reflex sympathetic dystrophy associated with Lyme disease^[7].

Given that immune dysfunction, postural orthostatic tachycardia syndrome, fatigue, cognitive dysfunction, orthostatic palpitations, syncope, and stress, which may occur in Lyme disease^[6-11], are associated with parasympathetic activity and reduced modulation of cardiac vagal tone (or the related measure of heart rate variability)^[4,12-14], we hypothesized that modulation of cardiac vagal tone might be impaired in this illness. The aim of our study was to test this hypothesis by comparing the response of cardiac vagal tone to respiratory modulation in a sample of Lyme disease patients and matched controls. To the best of our knowledge, this was the first such study.

MATERIALS AND METHODS

The arterial blood pressure, resting cardiac rate, resting cardiac vagal tone, and the cardiac vagal tone following deep breathing were measured in 18 serologically positive Lyme disease patients who were undergoing routine clinical investigation, and in 18 normal controls; all subjects were asked to refrain from any caffeine-containing beverages from midnight before testing. For inclusion in this study, the Lyme disease patients, who were selected from outpatient attendees, were required to be IgM positive for Lyme disease and to be aged 18 years or over. The control subjects were identified as subjects who were not suffering from any autonomic nervous system dysfunction (dysautonomia) or from any condition that might directly or indirectly affect the autonomic nervous system, and were recruited from hospital staff, and from friends and family of staff members. The exclusion criteria for the normal controls included: under 18 years of age; subjects with dysautonomia; taking medications that affect the autonomic nervous system, such as stimulants, tricyclic antidepressants, anti-histaminergic medication, calcium-channel blockers, beta-adrenoceptor blocking drugs, beta agonists, monoamine oxidase inhibitors, levodopa, anti-psychotic drugs, clonidine and vasopressin; medical problems that affect the autonomic nervous system, such as neurodegenerative disorders, peripheral neuropathies, diabetes, connective tissue disease and infectious diseases. The clinical stage and presentation of the patients were early, presumed localized and without cardiac involvement (that is, without clinical evidence of Lyme carditis). As there has been no previous work in this area, it was not possible to calculate the value of beta, and therefore

calculate the statistical power, for this study. Written informed consent was obtained and a local research ethics committee approved the study. The study was carried out in accordance with the Declaration of Helsinki.

Resting cardiac rate and cardiac vagal tone were measured in real time using the NeuroScope Model 300BA (Brainstem Autonomic Function Monitor) (Medifit Instruments Ltd, London, United Kingdom) as described by Little *et al.*^[15] and during a 10-s cycle of deep breathing as described by Julu *et al.*^[16]. In particular, the non-invasive cardiac vagal tone was measured on a continuous, beat-to-beat basis and was defined as pulse-synchronized phase shifts in consecutive cardiac cycles; it is essentially a form of pulse interval variability which is quantified continuously from the electrocardiogram. The index of cardiac vagal tone was measured and quantified in arbitrary units of a linear vagal scale; the minimum value of this scale is zero, which corresponds to full atropinisation of human subjects.

Arterial blood pressure was measured using the Ohmeda 2300 Finapres (Ohmeda, Englewood, CO, United States).

Continuous variables for which data did not differ significantly from normality and for which the two groups did not have significantly different variances were compared between the Lyme disease and control groups using independent samples *t*-tests (equal variances), while the discrete nominal variable (sex) was compared between groups using Fisher's exact probability test. The software package IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp, Armonk, NY, United States) was used for the statistical analyses.

RESULTS

The main findings are shown in Table 1. The two groups were matched in respect of age, sex, body mass, mean arterial blood pressure, mean resting heart rate, and mean resting cardiac vagal tone.

Details of the heart rate for each of the 18 patients before, during, and following the deep breathing procedure are provided in Table 2.

Corresponding details of the heart rate for each of the 18 control subjects before, during, and following the deep breathing are provided in Table 3.

The mean (\pm standard error) maximum cardiac vagal tone during deep breathing in the Lyme disease patients (11.2 ± 1.3) was significantly lower than that in the matched controls (16.5 ± 1.7 ; $P = 0.02$); these data are illustrated in Figure 1.

DISCUSSION

This study has demonstrated impairment of respiratory modulation of cardiac vagal tone in Lyme disease. This is an original finding which has not previously been described.

At the outset, it should be noted that our results demonstrate impaired respiratory modulation of cardiac vagal tone in Lyme disease; this is not the same as show-

Table 1 Main findings

	Lyme disease patients <i>n</i> = 18 mean (standard error)	Controls <i>n</i> = 18 mean (standard error)	<i>P</i> -value
Age, yr	35.6 (3.7)	44.7 (3.9)	0.10
Sex	7 males, 11 females	7 males, 11 females	1.00
Body mass	25.0 (1.2) kg	24.6 (0.80) kg	0.44
Arterial blood pressure	74.1 (3.9) mmHg	66.9 (4.4) mmHg	0.23
Resting cardiac rate	72.0 (3.1) min ⁻¹	63.7 (4.5) min ⁻¹	0.14
Resting cardiac vagal tone	4.7 (0.8)	5.7 (1.1)	0.46
Maximum cardiac vagal tone during deep breathing	11.2 (1.3)	16.5 (1.7)	0.02

Table 2 Heart rate data for the patients

Patient number	Heart rate at 20 beats before the deep breathing procedure	Heart rate 20 beats before the end of the deep breathing procedure	Heart rate two minutes after the deep breathing procedure
1	71.8	80.5	76.0
2	90.8	90.7	83.1
3	89.8	120.7	83.5
4	68.0	73.6	66.0
5	70.3	86.4	66.3
6	90.5	83.6	90.6
7	69.0	68.6	70.2
8	64.4	65.5	61.7
9	141.8	125.9	137.8
10	69.5	76.1	70.3
11	71.9	71.6	74.5
12	69.4	71.6	71.4
13	74.6	78.1	79.7
14	68.4	69.9	66.7
15	68.7	77.2	68.3
16	62.0	72.9	65.3
17	69.2	72.1	67.5
18	63.0	69.1	68.3

Table 3 Heart rate data for the controls

Patient number	Heart rate at 20 beats before the deep breathing procedure	Heart rate 20 beats before the end of the deep breathing procedure	Heart rate two minutes after the deep breathing procedure
1	86.8	88.7	90.9
2	52.8	70.2	52.0
3	67.0	74.9	69.5
4	73.7	83.7	75.8
5	62.9	63.9	54.3
6	65.0	73.4	61.8
7	65.2	69.2	65.6
8	73.8	78.0	76.0
9	64.5	70.6	53.8
10	66.6	66.7	64.2
11	63.4	72.6	66.7
12	62.9	69.8	63.1
13	75.4	73.5	79.0
14	78.8	81.8	72.1
15	70.6	81.2	66.4
16	76.0	70.9	74.9
17	88.0	92.3	81.8
18	75.9	74.8	68.3

ing changed cardiac vagal tone *per se*. Indeed, the resting cardiac vagal tone in the Lyme disease patients was found not to differ significantly from that in the matched control group. Therefore, in attempting to provide an explanation for our results, it will not suffice simply to look for causes of altered (*e.g.*, reduced) cardiac vagal tone in the

patient group.

The cause of the observed abnormalities might be vagal nerve changes resulting from Lyme disease. It is also worth bearing in mind that, since cardiac vagal tone is reflexly generated through arterial baroreceptor stimulation, by the afferents of the latter facilitating cardiac va-

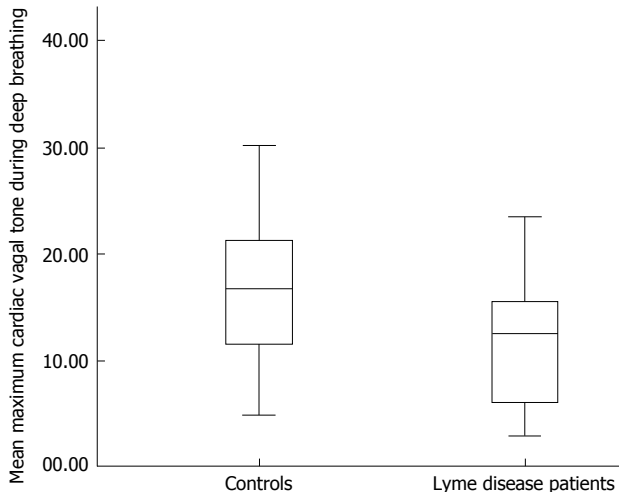


Figure 1 Boxplot of mean maximum cardiac vagal tone during deep breathing for the two groups.

gal motoneuron discharge relaying through interneurons in the nucleus tractus solitarius^[15,17,18], the results are also compatible with the possibility that Lyme disease might affect the brainstem. Indeed, given that the dorsal nucleus of the vagus nerve, the nucleus ambiguus, the nucleus tractus solitarius, and the spinal trigeminal nucleus, which give rise to or receive axons of the vagus nerve, are located in the medulla oblongata, these two possibilities are not mutually exclusive.

The causative *Borrelia* bacteria are able to undergo pleomorphic changes, including into a cystic form; indeed, it has been suggested that this may at least in part account for some cases of antibiotic resistance and recurrence of Lyme disease^[19]. It may be that this cystic form is often to be found in the brainstem in affected patients. However, this is unlikely to be an explanation here because this is believed to occur only in chronic Lyme neuroborreliosis.

It should be noted that there was no evidence that the patients were suffering from other disorders, such as Epstein-Barr viral infection, which might have caused false-positive serological results.

Finally, from Table 1 it can be seen that the mean resting cardiac rate in the controls (63.7 min^{-1}) was slightly (but not statistically significantly) lower than that in the patients (72.0 min^{-1}), while the mean resting cardiac vagal tone (5.7) was slightly (but not statistically significantly) higher than that in the patients (4.7). These findings might reflect the fact that the control group, in contrast to the Lyme patient group, were more physically fit; eight of the control subjects regularly engaged in exercise (sports, gymnasium attendance, or regular walking).

COMMENTS

Background

Lyme disease or Lyme borreliosis is an arthropod-borne zoonosis caused by *Borrelia* spirochetes, the incidence of which has recently been increasing with the geographical spread of infected ticks and which was previously identified clinically in Europe as Garin-Bujadoux-Bannwarth syndrome and in the United

States as Lyme arthritis.

Innovations and breakthroughs

Respiratory modulation of cardiac vagal tone is impaired in Lyme disease, which suggests that Lyme disease may directly affect the vagus nerve or the brainstem.

Applications

Cardiac vagal tone is reflexly generated through arterial baroreceptor stimulation, by the afferents of the latter facilitating cardiac vagal motoneuron discharge relaying through interneurons in the nucleus tractus solitarius, implying that Lyme disease may directly affect the vagus or brainstem.

Peer review

The topic is relatively new and there are few data about the topic of the study about (respiratory modulation of cardiac vagal tone in Lyme disease). This finding seems very interesting.

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Therapeutic equivalence in the treatment of hypertension: Can lercanidipine and nifedipine GITS be considered to be interchangeable?

Henry L Elliott, Peter A Meredith

Henry L Elliott, Institute of Pharmaceutical and Biomedical Sciences, University of Strathclyde, Glasgow G4 0RE, United Kingdom

Peter A Meredith, Department of Medicine and Therapeutics, University of Glasgow, The Western Infirmary, Glasgow G11 6NT, United Kingdom

Author contributions: Elliott HL and Meredith PA contributed equally to this work.

Correspondence to: Peter A Meredith, Senior University Teacher, Department of Medicine and Therapeutics, University of Glasgow, The Western Infirmary, 378 Sauchiehall Street, Glasgow G11 6NT,

United Kingdom. peter.meredith@glasgow.ac.uk

Telephone: +44-141-2112748 Fax: +44-141-2112748

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Abstract

AIM: To undertake a review of the evidence that nifedipine GITS and lercanidipine are therapeutically equivalent in the management of essential hypertension.

METHODS: A systematic review of the published literature was prompted by the findings of two meta-analyses which indicated that there was a lower incidence of peripheral (ankle) oedema with lercanidipine. However, neither meta-analysis gave detailed attention to comparative antihypertensive efficacy or cardiovascular protection. Accordingly, a systematic, detailed and critical review was undertaken of individual published papers. The review started with those studies incorporated into the 2 meta-analyses and then all other salient and directly relevant papers identified through the following search criteria: all randomized controlled trials in which the therapeutic profile and antihypertensive effects of lercanidipine were directly compared with those of nifedipine GITS (in hypertensive patients). The search

strategy was focused on the reports of clinical trials of lercanidipine *vs* nifedipine GITS, which were identified through a systematic search of PubMed (from 1966 to October 2012), Embase (from 1980 to October 2012) and the Cochrane library (from 1 October 2008 to end October 2013). The search combined terms related to lercanidipine *vs* nifedipine GITS (including MeSH search using calcium antagonists, calcium channel blockers and dihydropyridines).

RESULTS: With regard to blood pressure (BP) control and the consistency of BP control throughout 24-h, there is limited published evidence. However, two studies using 24 h ambulatory blood pressure monitoring clearly identified the dose-dependency of BP lowering with lercanidipine and its variably sustained 24-h efficacy. In contrast, there is evidence of a consistent antihypertensive effect throughout 24 h with nifedipine GITS. The incidence of the most common "side effect", *i.e.*, peripheral (ankle) oedema can be estimated as follows. For every 100 patients treated with lercanidipine, 2.5 will report oedema compared to 6 patients treated with nifedipine GITS. However, 98 or 99 patients will continue treatment with nifedipine GITS, compared with 99.5 patients on lercanidipine. Finally, with regard to outcome studies of cardiovascular (CV) morbidity and mortality, there is definitive outcome evidence for nifedipine GITS but there is no evidence that treatment with lercanidipine leads to reductions in CV morbidity and mortality.

CONCLUSION: There is no evidence in terms of long-term BP control and CV protection to justify the contention that lercanidipine is therapeutically equivalent to nifedipine GITS.

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Key words: Nifedipine GITS; Lercanidipine; Therapeutic

equivalence

Core tip: Even in this time of “evidence-based medicine”, there is a widespread presumption of “class effects” in therapeutic practice including that for antihypertensive drug treatments. Thus, guidelines tend to recommend treatment not with specific agents but with groups or classes such as “calcium channel blockers” on the presumption of the therapeutic equivalence or inter-changeability of different agents. This literature review focuses attention on the apparent therapeutic advantage of lercanidipine over nifedipine GITS on the basis of a lower incidence of the adverse effect of peripheral (ankle) oedema. Overall, however, the balance of evidence of efficacy favours nifedipine GITS.

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INTRODUCTION

Hypertension treatment guidelines, particularly those in Europe, recommend a long-acting dihydropyridine calcium channel blocker (CCB) in the routine management of patients with hypertension, either as first line monotherapy or as a suitable combination partner for all other types of antihypertensive drug^[1,2]. In general, however, the guidelines do not nominate individual agents and there is an overall presumption of a “class” effect, *i.e.*, there is a presumption of therapeutic equivalence amongst all dihydropyridine CCBs licensed for once daily administration. The picture is further complicated by the mechanism harnessed to attain the suitability for once daily administration^[3]. There have been three alternative approaches: (1) An intrinsic, extended elimination half life, as with amlodipine; (2) An “apparent” prolongation of half life *via* a sophisticated, modified release formulation, as with nifedipine GITS (Gastro-Intestinal Therapeutic System); and (3) An increased duration of action *via* increased membrane-binding characteristics (attributed to a high membrane partition coefficient) despite a relatively short elimination half life, as with lercanidipine and lacidipine.

Since direct, comparative outcome studies within a drug class are rare, therapeutic equivalence is usually assumed through an amalgamation of different types of evidence: for example, members of the same chemical family with similar pharmacological characteristics; comparisons of published papers which separately evaluate the drugs in question; comparative studies of the drugs, usually in parallel group designs, for surrogate end-points and adverse drug reactions (ADRs).

With regard to ADRs, peripheral (ankle) oedema is a well-recognised, dose-dependent “side effect” associated with chronic treatment with long-acting dihydropyridine CCBs such as nifedipine GITS, lercanidipine and amlo-

dipine. There remains some debate, however, about the relative incidence of peripheral oedema with each of these individual agents and, in particular, the claims of a lesser incidence with lercanidipine^[4,11]. There also is considerable doubt as to whether or not the balance between antihypertensive efficacy and tolerability is superior with lercanidipine.

The fundamental remit of this paper is a critical review of the published information relating to the comparisons of two dihydropyridine CCBs, nifedipine (in its GITS formulation: Gastro-Intestinal Therapeutic System) and lercanidipine. Such information has been derived from a limited number of published direct, head-to-head comparisons and a small number of additional publications from which relevant comparative information can be derived.

The question of therapeutic equivalence and the inter-changeability of the two drugs have been addressed under three sub-headings: (1) The fundamental pharmacological response: in this case, blood pressure reduction; (2) The profile of adverse drug reactions: in this case, peripheral oedema; and (3) The long term treatment benefits: in this case, cardiovascular protection.

MATERIALS AND METHODS

This review was conducted in three phases: Phase 1-The starting point was a meta-analysis published in August 2009 as a systematic review of randomised, controlled, comparative clinical trials published during all years through to August 2008^[6]; Phase 2-The second stage was a critical review of a second, updated meta-analysis published in 2011^[7]; and Phase 3-The third component was a systematic, detailed and critical review of individual papers. First, those studies incorporated into the 2 meta-analyses. Then, in addition, all other salient and directly relevant papers identified through the following search criteria: all randomized controlled trials in which the therapeutic profile and antihypertensive effects of lercanidipine were directly compared with those of nifedipine GITS (in hypertensive patients). The search strategy was focused on the reports of clinical trials of lercanidipine *vs* nifedipine GITS, which were identified through a systematic search of PubMed (from 1966 to October 2012), Embase (from 1980 to October 2012) and the Cochrane library (from 1 October 2008 to end October 2013). The search combined terms related to lercanidipine *vs* nifedipine GITS (including MeSH search using calcium antagonists, calcium channel blockers and dihydropyridines).

The reference lists of original reports and meta-analyses of studies involving dihydropyridine calcium antagonists (retrieved through the electronic searches) were also scrutinised to identify studies that might not have been included in the computerized databases.

RESULTS

Phase 1

For the purposes of this meta-analysis, lercanidipine

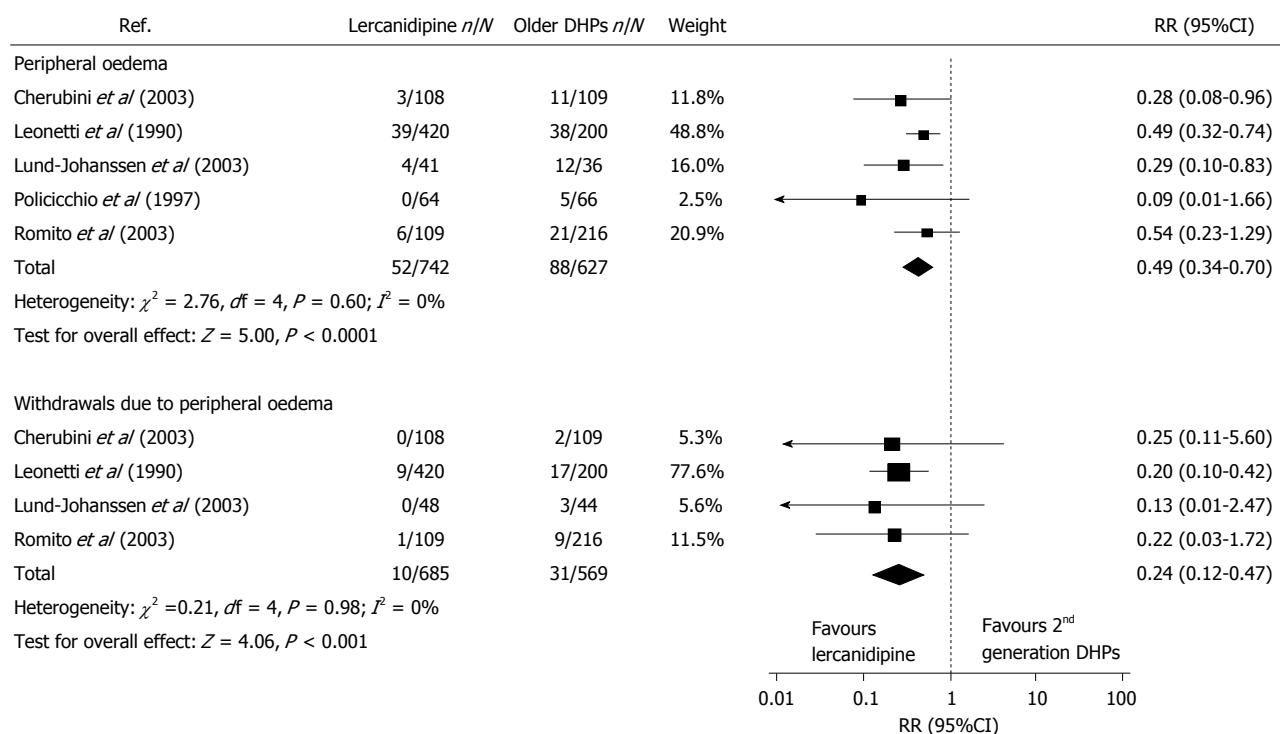


Figure 1 Incidence and withdrawals on account of peripheral oedema. Adapted and corrected from the first meta-analysis^[6].

was compared with a group of so-called “first generation dihydropyridine CCBs” (including nifedipine GITS and amlodipine)^[6]. The overall conclusion was that there was no significant difference between lercanidipine and these other competitor, “first generation CCBs” in terms of antihypertensive efficacy but there was a significant difference in favour of lercanidipine with respect to the incidence of, and withdrawal rates for, peripheral oedema (Figure 1).

Although three studies involving nifedipine GITS were cited in this paper, only 2 were included in the meta-analysis^[8-10]. It is important to note that within the statistical terms of the analysis itself, there were no significant differences between Nifedipine GITS and lercanidipine for the withdrawal rates for these 2 individual studies^[8,9] (Figure 1).

The 3 studies directly involving nifedipine GITS and lercanidipine are reviewed in greater detail below.

Phase 2

The second meta-analysis was more rigorous and more comprehensive but was essentially a repeat of the first analysis insofar as no new studies involving nifedipine GITS had been added^[7]. However, overall, it was a larger and more robust analysis by an independent group using stricter criteria.

In essence, the result was the same as for the first meta-analysis even although only 3 studies were incorporated for the comparison of lercanidipine and “older DHPs” (the same 2 studies with nifedipine GITS and a study involving amlodipine).

The conclusion was that, relative to “older DHPs”, lercanidipine was associated with a reduced incidence of

oedema: however, this component of the analysis was heavily influenced/weighted (78%) by the results of a study involving lercanidipine and amlodipine^[11]. Once again, within the structure of the meta-analysis, the same 2 individual studies with nifedipine GITS showed no statistically significant difference between nifedipine GITS and lercanidipine as far as the incidence of peripheral oedema was concerned (Figure 2)^[8,9].

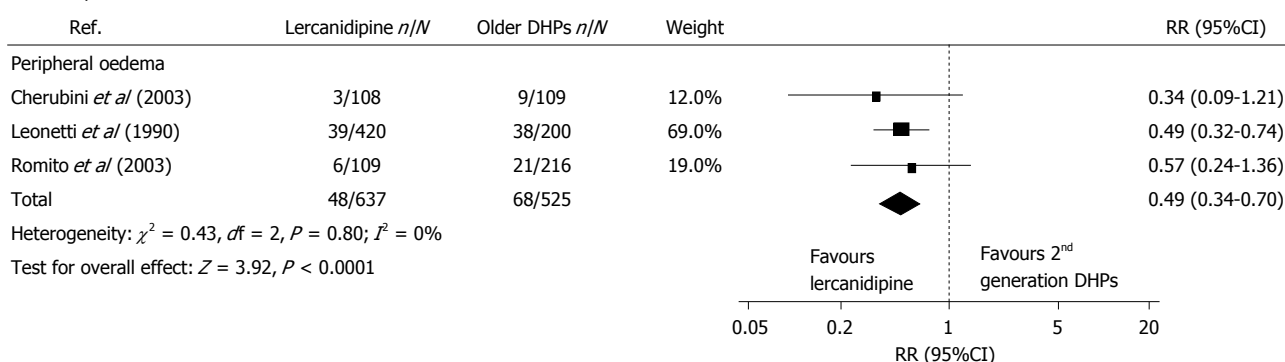
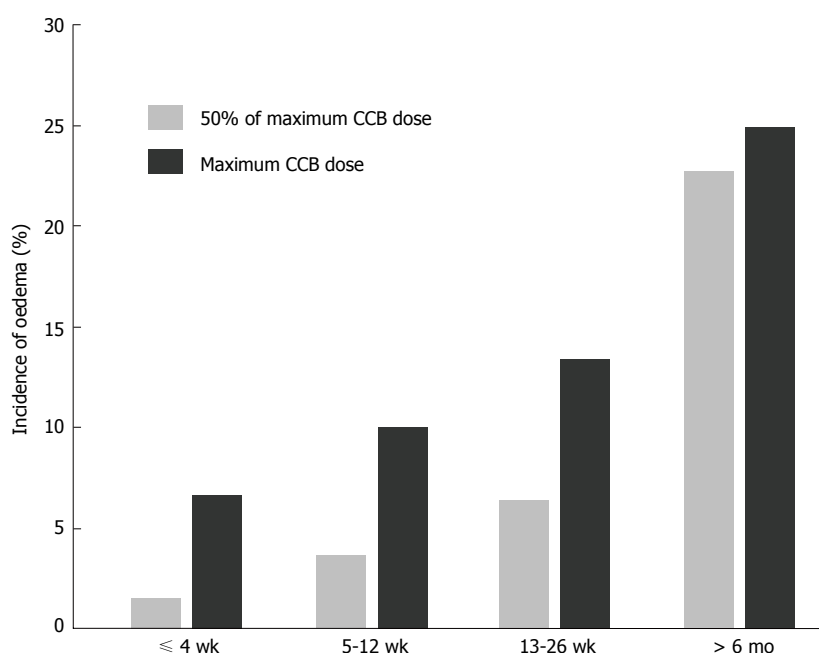
Additional features of clinical relevance and of practical importance in this second meta-analysis were as follows (Figure 3): (1) confirmation that the incidence of peripheral oedema is dose-dependent; (2) identification that the development of peripheral oedema is time-dependent, up to 6 mo treatment; and (3) awareness that the reduced incidence of peripheral oedema with “lipophilic DHPs” relative to “older DHPs” is not a unique feature of lercanidipine because lacidipine and manidipine were components of these analyses.

Phase 3-appraisal of individual studies

The comprehensive search of the literature databases revealed, in addition to the 3 studies cited in the first meta-analysis, a further 5 studies that met the pre-defined search criteria for the exploration of pertinent treatment issues.

Comparative studies: Romito *et al*^[9] reported a double blind, parallel group study of a total of 250 patients which compared lercanidipine (10 and 20 mg), felodipine (10 and 20 mg) and nifedipine GITS (30 and 60 mg) across an 8 wk treatment period. No significant differences in antihypertensive efficacy were reported.

The incidence of ADRs was significantly lower with

Lercanidipine *vs* older DHPsFigure 2 Incidence of peripheral oedema. Adapted from the second meta-analysis^[7].Figure 3 Dose and time-dependency of the development of peripheral oedema. Adapted from the second meta-analysis^[7].

both lercanidipine and nifedipine GITS: in particular, there were lower rates for ankle oedema with lercanidipine (5.5%) and nifedipine GITS (6.6%), relative to felodipine (13%). The incidence of ankle oedema was not significantly different for lercanidipine and nifedipine GITS.

Ankle oedema led to the withdrawal of 1 patient receiving lercanidipine ($n = 109$) compared to 4 patients receiving nifedipine GITS ($n = 106$) and 5 patients on felodipine ($n = 110$). These differences were not statistically significant.

Cherubini *et al*^[8] reported a double blind, randomised, parallel group study over 24 wk in elderly hypertensive patients comparing lacidipine (2 and 4 mg), lercanidipine (5 and 10 mg) and nifedipine GITS (30 and 60 mg).

The BP responder and normalisation rates were remarkably high with all 3 treatments, approximating to 100% in the case of nifedipine GITS, but not significantly different with the other 2 treatments. The incidence of oedema was lowest at 2.8% in the lercanidipine group

($n = 96$) compared to 7.5% with lacidipine ($n = 99$) and 10.1% with nifedipine GITS ($n = 97$) but this was not statistically significant. There were 2 withdrawals in the nifedipine group on account of oedema (out of 109 patients) and no withdrawals in the lercanidipine group.

There must be some concerns about the sensitivity of the BP methodology in this study because the BP responses were remarkably and unexpectedly high with all 3 drugs, especially considering that the doses of lacidipine and lercanidipine were relatively modest. With particular respect to lercanidipine, the consensus (in the published literature) is that 10 and 20 mg lercanidipine are the equivalent doses *vs* 30 and 60 mg nifedipine GITS. In fact, the 10/20 mg *vs* 30/60 mg comparability is specifically noted in the paper by Romito *et al*^[9].

Fogari *et al*^[10] designed specifically to assess indices of ankle volume and sub-cutaneous tissue pressure in patients randomly assigned in a double blind manner to 12 wk treatment with either lercanidipine (10 and 20 mg) or nifedipine GITS (30 and 60 mg).

There were no patient reports for peripheral oedema (hence the study was not incorporated into either of the published meta-analyses) and there were no patient withdrawals from either treatment group. There were statistically significant differences in ankle volume indices indicating a greater degree of ankle oedema with nifedipine GITS.

In summary, using a relatively sophisticated research methodology, this study demonstrated that nifedipine GITS had a greater propensity for the development of peripheral (ankle) oedema relative to lercanidipine. However, there were no clinical reports of any difference in incidence or withdrawal rates.

Other studies: Ambrosioni *et al.*^[12] reported the findings of 2 small studies exploring the antihypertensive efficacy of lercanidipine in doses of 5, 10 and 20 mg once daily in a total of 44 patients. Multiple 24-h ambulatory BP recordings were obtained and the following are the principal conclusions.

There was no statistically significant BP reduction with 5 mg lercanidipine but both single and multiple doses of 10 and 20 mg lercanidipine significantly reduced BP across 24 h. However, the BP reduction was not consistent across 24 h as assessed by the trough peak (TP) ratio. The TP ratios for the single dose study were not reported but, from one of the figures in the published paper, it can be estimated at about 39% with 10 mg and 44% with 20 mg; the corresponding values for multiple dosing were stated to be greater than 60%.

Omboni *et al.*^[13] reported a clinical study of more than 200 patients essentially confirmed the above findings: "At a dose of 10 mg lercanidipine had a significant and durable antihypertensive effect over 24 h but at 5 mg the effect was less consistent and did not last 24 h". For 10 mg lercanidipine the TP ratio was reported at above 60% and from one of the figures in the published paper it appears to fall into the range 60%-75%.

Borghi *et al.*^[14] reported an open label, sequential treatment study lasting for a total of 8 wk and reliant upon a BP measurement obtained at a single time point (not defined in relation to drug administration).

A total of 125 patients were entered into the study because they were known to be experiencing "calcium antagonist-specific ADRs", including peripheral oedema, whilst on treatment with amlodipine, felodipine, nifedipine GITS or nitrendipine. Patients were switched to lercanidipine and assessed after 4 wk treatment and then re-assigned to their original CCB and assessed again after a further 4 wk treatment.

In brief, no BP difference was detected (142/87 on lercanidipine and 141/87 mmHg on the other CCBs). Peripheral oedema was reported by 52% of patients after 4 wk of lercanidipine and by 87% of patients returned to their original CCB. The study did not report a direct comparison for the 28 patients treated with nifedipine GITS.

Once again, the BP methodology had little or no discriminatory power and the principal conclusion reflects a

comparison of lercanidipine against a group of "calcium antagonists", with amlodipine accounting for more than half of the patients.

Barrios *et al.*^[15] reported an observational study, conventional clinic BP control was significantly better (but of borderline clinical significance) at 144.4/83.3 in 233 patients receiving lercanidipine 20 mg daily, compared to 145.0/84.5 mmHg in 104 patients receiving either amlodipine 10 mg or nifedipine GITS 60 mg daily. However, and in addition to other methodological concerns, there was no direct comparison involving nifedipine GITS, nor any data on the number of patients receiving GITS. Furthermore, approximately 50% of the patient population were receiving concomitant antihypertensive drugs, with approximately 30% receiving 2 additional antihypertensive drugs.

DISCUSSION

Any interpretation of the available published literature is potentially compromised by issues relating to dosage equivalence, methodology, study reliability, statistical power, investigator bias, funding/sponsorship *etc.* Nevertheless, the following is presented as an objective summary of the available evidence evaluating whether or not lercanidipine and nifedipine GITS can be considered to be therapeutically equivalent for the management of hypertension.

Antihypertensive efficacy

The initial report of antihypertensive equivalence reflected the achieved BPs at 24 h post-dose in the 3 comparative studies cited in the original meta-analysis^[6], *i.e.*, there were no statistically significant differences. However, this interpretation not only reflected a rather insensitive measure of overall BP control but also raised concerns because: (1) one of these studies was not designed as a BP comparison; and (2) a second study had clear methodological flaws because it assigned a responder rate of 86% for 5mg lercanidipine, a dose which 2 other studies found to be inadequately effective. Thus, there is a very "thin" evidence base for direct antihypertensive equivalence if reliance is placed upon conventional clinic BP measurements.

There obviously are other clinical reports which assessed the antihypertensive efficacy of lercanidipine but these were not direct, head-to-head comparisons. Overall, whilst equivalence (with nifedipine GITS) was inferred on the basis of results which were similar, these results cannot be directly compared in statistical terms because they were generated by different research groups, in different patient populations, using different methodologies, *etc.* There also are publications in which deductions are made in spite of confounding factors and the TOLERANCE study is an illustrative example^[15]. As discussed above, there was no direct comparison of lercanidipine and nifedipine GITS in this study, nor any data on the number of patients receiving GITS, and approximately 50% of the patient population were receiving at least one other antihypertensive drug.

In the absence of any other evidence, it might have proved difficult to challenge the conclusion of antihypertensive equivalence (which is actually based on one single, relatively robust, direct comparison!) but there were 2 studies employing 24 h ambulatory BP monitoring which explored the antihypertensive efficacy of lercanidipine in greater detail^[12,13]. Both studies identified the dose-dependency of the 24 h BP lowering effects with lercanidipine which, overall, displayed poorly sustained 24-h efficacy. Additionally these 2 papers incorporated measurements of trough: TP as part of their assessments of antihypertensive efficacy throughout 24 h. Whilst TP ratio as an index of antihypertensive efficacy is not without its limitations^[16], values of respectively 39% and 44% (estimated) following single 10 and 20 mg doses of lercanidipine and of “greater than 60%” for both doses during steady state treatment are not particularly high. In contrast, the published data with nifedipine GITS (by the same group, using the same methodology as for one of the lercanidipine studies) demonstrated that the drug elicits a consistent antihypertensive effect that is independent of dose and is characterised by a TP ratio approximating to 100%^[17].

In summary, it may be reasonably concluded that BP control throughout 24-h is more consistent and better sustained with nifedipine GITS than with lercanidipine during chronic treatment.

Peripheral (ankle) oedema

Despite the paucity of direct comparative studies, there is a reasonable volume of evidence to indicate that this “side effect” is less likely to occur with lercanidipine than with nifedipine GITS.

In the absence of definitive statistics, a reasonable approximation of the practical consequences of this differentiating characteristic is derived from the second meta-analysis which incorporated data from 99469 patients. In brief, for every 100 patients treated with lercanidipine, 2.5 will report peripheral oedema compared to 6 patients treated with nifedipine GITS: correspondingly, 0.5 patients will withdraw from lercanidipine treatment compared to 1.1 treated with nifedipine GITS. The corollary of this is that there will be 98 or 99 patients continuing treatment with nifedipine GITS, compared with 99.5 patients treated with lercanidipine.

As a footnote, however, there is the suggestion that this potentially advantageous feature is not unique to lercanidipine insofar as it may also be a feature of treatment with lacidipine and manidipine.

Cardiovascular protection

For nifedipine GITS there is definitive evidence of benefit in the treatment of hypertension and stable coronary artery disease^[18,19]. As there are no clinical outcome studies, there is no evidence that treatment with lercanidipine leads to reductions in cardiovascular (CV) morbidity and mortality.

In summary, the available evidence confirm the claims that lercanidipine has a lesser incidence of peripheral (ankle) oedema, relative to treatment with nifedipine

GITS. Whilst this may be factually accurate, it is a trivial difference in terms of clinical practice, particularly with respect to patient withdrawals, whereby 98 patients (out of 100) will continue treatment with nifedipine GITS. Set against this minor advantage and albeit with incomplete evidence, the antihypertensive efficacy of nifedipine GITS appears to be superior, particularly in respect of sustained 24-h BP control.

In conclusion, the ultimate aim of antihypertensive drug treatment (with CCBs and other classes of drugs) is sustained, long term BP control leading to a significant reduction in CV morbidity and mortality. There is no compelling evidence with lercanidipine to undermine the proven ability of nifedipine GITS to reduce death and CV events on the basis of an occasional, inconvenient but essentially innocuous adverse effect. Thus, there is no justification for assuming therapeutic equivalence between lercanidipine and nifedipine GITS and no grounds for considering that they are interchangeable if CV protection is the ultimate goal.

COMMENTS

Background

Drugs in the same “class” are often considered to be therapeutically equivalent and, therefore, inter-changeable. This review scrutinises the published literature to compare two antihypertensive drugs (lercanidipine and nifedipine GITS) and to assess whether or not there is good evidence that these drugs are therapeutically similar.

Research frontiers

Several new guidelines from international organisations have been recently published to advise on the treatment of hypertension but, as a general rule, no guidance is given on the choice of individual drugs. This critical review is intended for the prescribing clinician to allow him or her to make an informed evidence-based assessment which, in this case, addresses the comparison of two calcium channel blocking drugs.

Applications

The practical conclusion is that lercanidipine and nifedipine GITS cannot be considered to be inter-changeable because, although it appears less likely to cause peripheral (ankle) oedema, there is no direct evidence that lercanidipine can provide cardiovascular protection. In contrast, there is clear evidence of consistent 24-h blood pressure control and long-term cardiovascular protection with nifedipine GITS.

Peer review

This is an interesting paper. The authors have reviewed the role of lercanidipine in hypertension and compared it to nifedipine GITS in an extensive manner.

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Headache: An unusual presentation of acute myocardial infarction

Dimitrios Asvestas, Konstantinos Vlachos, Anastasios Salachas, Konstantinos P Letsas, Antonios Sideris

Dimitrios Asvestas, Konstantinos Vlachos, Anastasios Salachas, Konstantinos P Letsas, Antonios Sideris, Second Department of Cardiology, Evangelismos General Hospital, 10676 Athens, Greece

Author contributions: Asvestas D reviewed the literature, and helped to draft the manuscript; Vlachos K reviewed the literature, and helped to draft the manuscript; Salachas A performed the coronary angiography; Letsas KP critically revised the manuscript; Sideris A critically revised the manuscript; all authors have read and approved the final manuscript.

Correspondence to: Konstantinos P Letsas, MD, FESC, Second Department of Cardiology, Evangelismos General Hospital, Ipsilantou 45-47, 10676 Athens, Greece. k.letsas@mail.gr

Telephone: +30-21-07201466 Fax: +30-21-32041344

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rule out the possibility of hemorrhage.

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INTRODUCTION

Atypical symptoms of myocardial infarction may delay the diagnosis, and therefore the proper management to rescue ischemic myocardium. Headache represents a rare symptom of myocardial ischemia^[1-5]. We report a patient with ST-segment elevation acute myocardial infarction who presented to the emergency department complaining of headache without chest discomfort.

CASE REPORT

An 86-year-old man with a history of hypertension and tobacco use presented to the emergency department complaining of recent onset severe occipital headache. The patient did not report any chest pain, dyspnea, or other typical symptoms of angina. On admission the patient was pale with tachycardia (100 beats/min), and, while his blood pressure was within normal range (100/60 mmHg). At auscultation, a mild systolic murmur was audible. The electrocardiogram (ECG) showed sinus bradycardia, ST-segment depression in leads V1-V5 and ST-segment elevation in posterior leads (V7-V9) (Figure 1). Transthoracic echocardiography revealed an impaired left ventricular ejection fraction (40%-45%) along with mild mitral valve regurgitation. Initial laboratory examinations showed elevated levels of high-sensitivity cardiac troponin T (250 ng/L). Due to his clinical presentation, a brain computed tomography (CT) imaging was im-

Abstract

Acute myocardial infarction should be diagnosed as early as possible for the appropriate management to salvage ischemic myocardium. Accurate diagnosis is typically based on the typical symptoms of angina. Headache is an unusual symptom in patients with acute myocardial infarction. We report a patient with ST-segment elevation acute myocardial infarction who presented to the emergency department complaining of severe occipital headache without chest discomfort.

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Key words: Headache; Angina; Myocardial infarction

Core tip: The association of headache with myocardial ischemia is unusual and is accompanied by chest discomfort. The only symptom of this patient was occipital headache and this is extremely rare. Owing to the rare occurrence of headache as a symptom of myocardial ischemia, diagnosis may be extremely difficult since a brain computed tomography imaging is important to

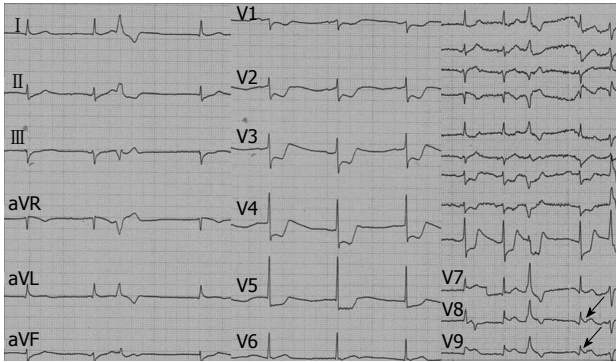


Figure 1 Electrocardiogram on admission demonstrating ST-segment depression in leads V1-V5 and ST-segment elevation in the posterior leads (V7-V9) (arrows).

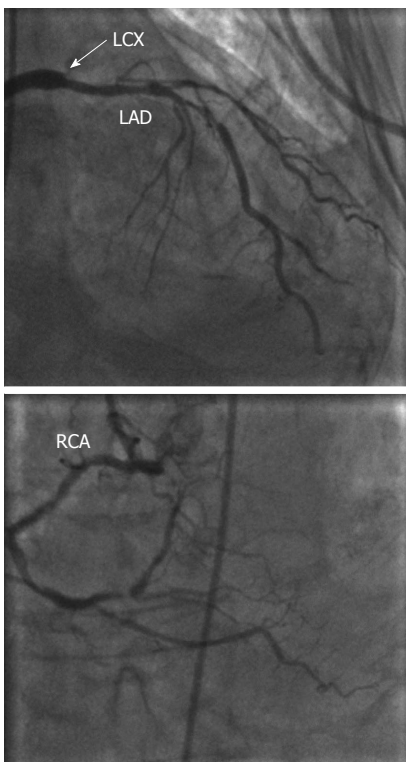


Figure 2 Coronary angiography showing total obstruction of the proximal left circumflex artery (arrow) and severe stenosis in left anterior descending artery and right coronary artery. LCX: Left circumflex artery; LAD: Left anterior descending artery; RCA: Right coronary artery.

mediately performed. The CT imaging was negative for intracerebral or subarachnoid hemorrhage. Following CT imaging, the patient prepared for cardiac catheterization and received aspirin (500 mg), clopidogrel (600 mg) and unfractionated heparin (70 U/kg). Coronary angiography was performed 60 min after admission and demonstrated a three-vessel coronary artery disease [the proximal left circumflex artery (LCX) was totally obstructed, the left anterior descending artery (LAD) displayed a severe stenosis and the right coronary artery was also severely diseased] (Figure 2). Proximal LAD lesion was directly stented, while the blood flow was restored in LCX artery revealing a severe stenosis of more than

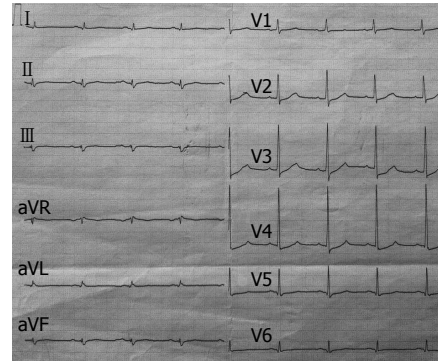


Figure 3 Electrocardiogram demonstrating resolution of the ST-segment depression in leads V1-V5 after revascularization.

90%. We attempted to insert the guidewire into the LCX but failed to cross the proximal part of LCX. Following revascularization, the patient was totally asymptomatic without headache, while the ECG was normalized (Figure 3). During the following days, the myocardial enzymes (CK-MB, hs-troponin T) followed the classic rise and fall kinetic pattern. He discharged 6 d later under dual anti-platelet (aspirin, clopidogrel), β -blocker and angiotensin converting enzyme inhibitor therapy.

DISCUSSION

Myocardial infarction should be diagnosed as early as possible for the appropriate management to salvage ischemic myocardium. Accurate diagnosis is based on both ECG and clinical presentation of the patient. Ischemia and myocardial infarction typically causes chest pain variously radiating elsewhere (shoulders, upper extremities and epigastrium). The association of headaches with myocardial ischemia is unusual and is accompanied by chest discomfort. The only symptom of this patient was occipital headache and this is extremely rare. Owing to the rare occurrence of headache as a symptom of myocardial ischemia, diagnosis may be extremely difficult since a brain CT imaging is important to rule out the possibility of hemorrhage.

The incidence of headache as a symptom of myocardial ischemia may be underestimated^[1-5]. Culic *et al*^[6] reported that headache is present (along with other symptoms) in 5.2% of patients with acute myocardial infarction. Moreover, in 3.4% of these patients headache was the primary complaint^[6]. Cardiac cephalalgia or headache angina is a recognized phenomenon, but the pathophysiological mechanism is still unclear^[7-8]. There is a connection between the central cardiac pathway and the cranial pain afferents. The cardiac sympathetic fibers originate from cervical lymph nodes which also innervate pain sensitive cranial structures^[9-10]. Furthermore, it is hypothesized that chemical mediators like bradykinin, serotonin and histamine can induce pain in shoulders, arms, neck and in this case headache. Another mechanism is based on the elevated intracranial pressure associated in the case of decreased cardiac output during myocardial

infarction and elevated venous pressure^[11,12]. Finally, increased levels of atrial and brain natriuretic peptides may be involved in intracranial pressure regulation^[13]. Even though the occurrence of headache as a sole manifestation of angina or myocardial infarction has been previously described, many clinicians ignore this unusual manifestation. The diagnosis of “cardiac headache” is difficult and requires a high degree of suspicion.

COMMENTS

Case characteristics

An 86-year-old man presented to the emergency department complaining of recent onset severe occipital headache.

Clinical diagnosis

The patient was pale with tachycardia and electrocardiogram (ECG) signs suggestive of myocardial infarction.

Differential diagnosis

The differential diagnosis included intracerebral or subarachnoid hemorrhage and myocardial infarction.

Laboratory diagnosis

Elevated levels of high-sensitivity cardiac troponin T were initially recorded.

Imaging diagnosis

Brain computed tomography imaging excluded intracerebral or subarachnoid hemorrhage, while coronary angiography demonstrated a three-vessel coronary artery disease.

Treatment

Proximal left anterior descending artery lesion was directly stented, while the blood flow was restored in left circumflex artery revealing a severe stenosis of more than 90%.

Experiences and lessons

Careful ECG interpretation in the setting of acute headache is of major importance.

Peer review

Asvestas *et al* report a rare case of a patient who presented with headache as the sole symptom of an acute myocardial infarction. The mechanisms by which headache is linked to ischemic vascular disease remain uncertain and are likely to be complex. The paper is generally well-written and interesting.

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World Journal of *Cardiology*

World J Cardiol 2014 July 26; 6(7): 517-691



TOPIC HIGHLIGHT

- 517 Antihypertensive drugs and glucose metabolism
Rizos CV, Elisaf MS
- 531 Hypertension and medical expenditure in the Japanese population: Review of prospective studies
Nakamura K, Okamura T, Miura K, Okayama A
- 539 Adipose tissue and vascular inflammation in coronary artery disease
Golia E, Limongelli G, Natale F, Fimiani F, Maddaloni V, Russo PE, Riegler L, Bianchi R, Crisci M, Di Palma G, Golino P, Russo MG, Calabrò R, Calabrò P
- 555 Risks and diagnosis of coronary artery disease in Hodgkin lymphoma survivors
Kupeli S
- 562 Non-invasive evaluation of arrhythmic risk in dilated cardiomyopathy: From imaging to electrocardiographic measures
Iacoviello M, Monitillo F
- 577 Mechanisms underlying the impaired contractility of diabetic cardiomyopathy
Ward ML, Crossman DJ
- 585 Distribution of late gadolinium enhancement in various types of cardiomyopathies: Significance in differential diagnosis, clinical features and prognosis
Satoh H, Sano M, Suwa K, Saitoh T, Nobuhara M, Saotome M, Urushida T, Katoh H, Hayashi H
- 602 Takotsubo cardiomyopathy: Pathophysiology, diagnosis and treatment
Komamura K, Fukui M, Iwasaku T, Hirotsu S, Masuyama T
- 610 Stem cell mechanisms during left ventricular remodeling post-myocardial infarction: Repair and regeneration
Zamilpa R, Navarro MM, Flores I, Griffey S
- 621 Chronic total occlusion: To treat or not to treat
Bardaji A, Rodriguez-López J, Torres-Sánchez M

- 630 Significance of lead aVR in acute coronary syndrome
Tamura A

REVIEW

- 638 Calpain system and its involvement in myocardial ischemia and reperfusion injury
Neuhof C, Neuhof H
- 653 Neuregulin-1/erbB activities with focus on the susceptibility of the heart to anthracyclines
Vasti C, Hertig CM

MINIREVIEWS

- 663 Assessment of coronary artery disease using coronary computed tomography angiography and biochemical markers
Gitsioudis G, Katus HA, Korosoglou G

CASE CONTROL STUDY

- 671 Lipid profile in children with coronary artery disease in Sindh, Pakistan
Baloch S, Devrajani BR, Baloch MA, Pir MA

RETROSPECTIVE STUDY

- 675 Is cardiac MRI an effective test for arrhythmogenic right ventricular cardiomyopathy diagnosis?
Chellamuthu S, Smith AM, Thomas SM, Hill C, Brown PWG, Al-Mohammad A

CASE REPORT

- 682 Rare case of coronary to pulmonary vein fistula with coronary steal phenomenon
Barsoum EA, Saiful FB, Asti D, Morcus R, Khoeiry G, Lafferty J, McCord DA
- 685 Worsening of coronary spasm during the perioperative period: A case report
Teragawa H, Nishioka K, Fujii Y, Idei N, Hata T, Kurushima S, Shokawa T, Kihara Y

LETTER TO THE EDITOR

- 689 3D-echo in preoperative assessment of aortic cusps effective height
Nijs J, Gelsomino S, Kietselaer BBLJH, Parise O, Lucà F, Maessen JG, La Meir M

Contents

World Journal of Cardiology
Volume 6 Number 7 July 26, 2014

APPENDIX I-V Instructions to authors

ABOUT COVER Editorial Board Member of *World Journal of Cardiology*, Manel Sabate, MD, PhD, Adjunct Professor, Cardiology Department, Clinic University Hospital, Barcelona 08036, Spain

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WJC 6th Anniversary Special Issues (1): Hypertension

Antihypertensive drugs and glucose metabolism

Christos V Rizos, Moses S Elisaf

Christos V Rizos, Moses S Elisaf, Department of Internal Medicine, School of Medicine, University of Ioannina, 45110 Ioannina, Greece

Author contributions: Rizos CV and Elisaf MS contributed equally to this paper.

Correspondence to: Moses S Elisaf, MD, FASA, FRSH, Professor, Department of Internal Medicine, School of Medicine, University of Ioannina, Ipiros, 45110 Ioannina, Greece. egepi@cc.uoi.gr

Telephone: +30-26510-07509 Fax: +30-26510-07016

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Abstract

Hypertension plays a major role in the development and progression of micro- and macrovascular disease. Moreover, increased blood pressure often coexists with additional cardiovascular risk factors such as insulin resistance. As a result the need for a comprehensive management of hypertensive patients is critical. However, the various antihypertensive drug categories have different effects on glucose metabolism. Indeed, angiotensin receptor blockers as well as angiotensin converting enzyme inhibitors have been associated with beneficial effects on glucose homeostasis. Calcium channel blockers (CCBs) have an overall neutral effect on glucose metabolism. However, some members of the CCBs class such as amlodipine and nifedipine have been shown to have advantageous effects on glucose homeostasis. On the other hand, diuretics and β -blockers have an overall disadvantageous effect on glucose metabolism. Of note, carvedilol as well as nebivolol seem to differentiate themselves from the rest of the β -blockers class, being more attractive options regarding their effect on glucose homeostasis. The adverse effects of some blood pressure lowering drugs on glucose metabolism may, to an extent, compromise their cardiovascular protective role. As a result the effects on glucose homeostasis of the various blood pressure lowering drugs should be taken into account when

selecting an antihypertensive treatment, especially in patients which are at high risk for developing diabetes.

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Key words: Hypertension; Glucose metabolism; Antihypertensive drugs

Core tip: Hypertension is a major contributor to the development and progression of cardiovascular disease. Increased blood pressure often coexists with insulin resistance. The various antihypertensive drugs have different effect on glucose metabolism. Indeed, angiotensin receptor blockers as well as angiotensin converting enzyme inhibitors have been associated with beneficial effects on glucose homeostasis. Calcium channel blockers are considered to have neutral metabolic effects. On the other hand, diuretics and β -blockers have an overall disadvantageous effect on glucose metabolism. As a result the metabolic effects of the various blood pressure lowering drugs should be taken into account when selecting an antihypertensive treatment.

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INTRODUCTION

Hypertension is a growing epidemic affecting an important percentage of the population^[1]. Hypertensive patients have increased risk for the development and progression of both microvascular and macrovascular complications. As a result the need for a comprehensive management of high blood pressure is essential.

Hypertension is strongly associated with risk factors that impair glucose homeostasis and is often presented as a component of the metabolic syndrome. Indeed,

hypertension is related with obesity, insulin resistance as well as diabetes mellitus^[2,3]. As a result, hypertensive patients have a 2.5-fold higher risk of type 2 diabetes mellitus (T2DM) onset compared with normotensive subjects^[4]. The various classes of antihypertensive drugs have different effects on blood glucose metabolism. Indeed, antihypertensive agents, such as β -blockers and thiazide diuretics have been associated with negative effects on blood glucose in contrast to other classes, such as angiotensin receptor blockers (ARBs) and angiotensin converting enzyme inhibitors (ACE-I). As a result, the treatment of hypertensive subjects should be carefully selected as to not further deteriorate an already at risk glucose homeostasis.

RESEARCH

We searched PubMed up to December 2013 using combinations of the following keywords: hypertension, glucose metabolism, glucose homeostasis, antihypertensive drugs, angiotensin converting enzyme inhibitors, ARBs, calcium channel blockers (CCB), β blockers, renin inhibitors, alpha blockers, diuretics. Major randomized controlled trials, original papers, review articles and case reports were included. The references of these articles were scrutinized for relevant articles. For articles not written in English, only the abstracts were considered. A minor limitation of this review is that our literature search was exclusively based on the PubMed database.

RENIN ANGIOTENSIN ALDOSTERONE SYSTEM

The renin angiotensin aldosterone system (RAAS) is strongly associated with glucose homeostasis. A number of studies have identified antihypertensive drugs that act by intervening in the RAAS as overall having beneficial effects on glucose metabolism.

Angiotensin converting enzyme inhibitors

The majority of clinical trials evaluating the effects of ACE-I on glucose metabolism have showed a positive outcome. Large clinical trials have revealed that ACE-I are associated with a lower incidence of new-onset T2DM in hypertensive subjects. The heart outcomes prevention evaluation (HOPE) study demonstrated the favorable influence of ramipril on cardiovascular (CV) disease (CVD) incidence in high risk patients^[5]. Patients recruited were ≥ 55 years old, had a history of coronary artery disease, stroke, peripheral vascular disease, or diabetes plus at least one other CV risk factor [hypertension, elevated total cholesterol levels, low high-density lipoprotein cholesterol (HDL-C) levels, cigarette smoking, or documented microalbuminuria]. For a mean period of 5 years the HOPE trial randomized the above high-risk patients ($n = 9279$) to ramipril (10 mg/d) or placebo. Ramipril reduced new onset diabetes by 34% ($P < 0.001$ *vs* placebo)^[5]. However, there are some limitations of

the HOPE results regarding new onset diabetes. Indeed, diabetes development in HOPE was not a pre-specified endpoint of the study. Moreover, the diagnosis of diabetes was patient reported.

Similarly, the Captopril Prevention Project (CAPPP) study was a prospective, randomized trial which compared the effect of captopril *vs* antihypertensive treatment with diuretics, β -blockers, or both in hypertensive patients ($n = 10985$)^[6]. Treatment with captopril was associated with fewer patients developing diabetes compared with the control group (OR = 0.79; 95%CI: 0.67-0.94; $P = 0.007$). However, because of the design of the study, the query arises as to whether the differences in development of T2DM in the CAPPP trial were due to a protective effect of ACE-I or a deleterious effect of β -blockers and diuretics. Another limitation of the study was that blood pressure as well as diabetes mellitus at baseline was more common in the captopril group than in the group that received conventional treatment. In addition, in the captopril group a diuretic or a CCB was added to treatment in order to achieve the blood pressure goal.

The Antihypertensive and Lipid-Lowering Treatment to prevent heart attack trial (ALLHAT) was a randomized, double-blind, trial which evaluated whether treatment with a CCB or an ACE-I lowers the incidence of coronary heart disease (CHD) or other CVD events *vs* treatment with a diuretic^[7]. Patients ($n = 33357$) with hypertension and at least one other cardiac heart disease risk factor were randomized to chlorthalidone, amlodipine, or lisinopril for a mean follow-up of 4.9 years. Lisinopril treatment reduced the relative risk of developing T2DM by 30% (95%CI: 23%-37%; $P < 0.001$) compared with patients treated with chlorthalidone and by 17% (95%CI: 7%-26%; $P < 0.01$) compared with patients treated with the amlodipine^[7].

The studies of left ventricular dysfunction (SOLVD) was a double-blind trial which randomized patients with asymptomatic left ventricular (LV) dysfunction to receive enalapril or placebo for a mean follow-up of 37.4 mo^[8]. Enalapril significantly reduced the incidence of heart failure and the rate of related hospitalizations compared with placebo^[8]. A retrospective study evaluated the effect of enalapril on the incidence of diabetes in patients from the SOLVD trial^[9]. Enalapril significantly reduced the incidence of diabetes compared with placebo (HR = 0.22; 95%CI: 0.10-0.46; $P < 0.0001$)^[9].

On the other hand, some studies have shown that ACE-I have a neutral effect on glucose metabolism. A study in patients with T2DM and hypertension ($n = 24$) resulted in no change in insulin sensitivity aftertrandolapril treatment^[10]. Similarly, enalapril treatment did not affect insulin sensitivity in patients with essential hypertension ($n = 20$)^[11]. Moreover, lisinopril did not affect insulin sensitivity in healthy volunteers ($n = 22$)^[12]. The Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) study evaluated the effects of ramipril or placebo in patients ($n = 5269$) without CVD but with impaired fasting glucose levels or impaired glucose tolerance. Patients received ramipril (up to 15 mg

per day) or placebo (and rosiglitazone or placebo) for a median of 3 years^[13]. Although ramipril treatment did not reduce the incidence of diabetes, it increased regression to normoglycemia in the study population ($P = 0.001$). The Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication Ongoing Follow-up (DREAM On) study followed patients from the DREAM trial for a median 1.6 years after the end of the trial^[14]. Ramipril did not influence diabetes occurrence. Similarly, regression to normoglycemia was not altered by ramipril.

A meta-analysis of randomized control trials associated ACE-I treatment with a reduction of new-onset T2DM (RR = 0.73; 95%CI: 0.63-0.84)^[15]. Similar were the results of another meta-analysis of randomized clinical trials where ACE-I had a smaller incidence of new-onset T2DM (OR = 0.77; 95%CI: 0.72-0.82; $P < 0.0006$) compared with control groups^[16].

ARBs

Treatment with ARBs has also been associated with an overall beneficial effect on glucose homeostasis. Indeed, large clinical trials have associated ARB treatment with lower incidence of new-onset T2DM. The losartan intervention for endpoint reduction (LIFE) in hypertension study was a double-blinded, randomized, parallel-group trial in patients ($n = 9193$) aged 55-80 years with essential hypertension (sitting blood pressure 160-200/95-115 mmHg) and LV hypertrophy^[17]. Patients were randomized to losartan or atenolol based antihypertensive treatment for a mean follow-up of 4.8 years^[17]. Losartan treatment was associated with a reduction of new-onset T2DM compared with the control group (HR = 0.75; 95%CI: 0.63-0.88; $P = 0.001$).

The Antihypertensive treatment and Lipid Profile In a North of Sweden Efficacy Evaluation (ALPINE) study compared the effect of hydrochlorothiazide, alone or in combination with atenolol, against candesartan, alone or in combination with felodipine, in newly diagnosed patients with primary hypertension ($n = 342$)^[18]. After 12 mo, fasting plasma glucose and fasting serum insulin increased in the diuretic group, while a decrease was observed in the candesartan group ($P < 0.001$ for the comparison of the 2 groups). The incidence of new-onset T2DM was higher in the hydrochlorothiazide (4.1%) group compared with the candesartan group (0.5%; $P = 0.03$)^[18].

The Valsartan Antihypertensive Long-term Use Evaluation (VALUE) was a prospective, double-blind, randomized trial that recruited hypertensive patients with additional CV risk factors^[19]. Study subjects were randomized to either valsartan or amlodipine based regimen. Drug up-titration or the addition of further antihypertensive drugs, excluding ARBs, was allowed to achieve BP control. The valsartan based group had a smaller incidence of new-onset T2DM compared with the amlodipine group (HR = 0.77; 95%CI: 0.69-0.86; $P < 0.0001$)^[19].

The Candesartan in Heart failure Assessment of Reduction in Mortality and morbidity (CHARM) study was a double-blind randomized control trial which

evaluated candesartan *vs* placebo in patients with heart failure ($n = 7599$) for a median follow-up of 37.7 mo^[20]. Among patients without a history of diabetes, new-onset T2DM was significantly lower in the candesartan group compared with the placebo group (HR = 0.78; 95%CI: 0.64-0.96; $P = 0.020$)^[20]. The CHARM program consisted of 3 component trials, each comparing candesartan with placebo in a distinct population of patients with symptomatic heart failure: (1) the CHARM-Alternative which included patients with LV ejection fraction (LVEF) $\leq 40\%$ and intolerant of ACE-I; (2) the CHARM-Added which included patients with LVEF $\leq 40\%$ who were treated with an ACE-I; and (3) the CHARM-Preserved which included patients with LVEF $> 40\%$. The candesartan group had a smaller incidence of T2DM compared with placebo only in the CHARM-Preserved trial (OR = 0.60; 95%CI: 0.41-0.86; $P = 0.005$).

The nateglinide and valsartan in impaired glucose tolerance outcomes research (NAVIGATOR) was a double-blind, randomized clinical trial in subjects with impaired glucose tolerance with known CVD or with CV risk factors^[21]. Patients ($n = 9518$) were randomized to receive valsartan (up to 160 mg daily) or placebo for a median of 5.0 years. The valsartan group had a smaller incidence of T2DM compared with placebo (HR = 0.86; 95%CI: 0.80-0.92; $P < 0.001$)^[21]. Despite the reduction of T2DM incidence, valsartan treatment did not reduce the rate of CV events.

On the other hand, the Study on Cognition and Prognosis in the Elderly (SCOPE) evaluated the effects of candesartan *vs* placebo in elderly patients aged 70-89 years ($n = 4964$) with hypertension for a mean follow-up of 3.7 years^[22]. Open-label active antihypertensive therapy was added as needed. There was not a significant difference regarding new-onset T2DM between the 2 groups^[22]. Similarly the CHARM-Added as well as the CHARM-Alternative studies did not show a difference regarding new-onset T2DM with candesartan treatment^[20].

A number of meta-analyses indicate the protective role of ARB treatment regarding T2DM development. Geng *et al*^[23] in a meta-analysis of 11 randomized control trials with 79773 patients (59862 non-diabetic patients at baseline) showed a beneficial effect of ARBs on T2DM development. Incidence of new-onset diabetes was significantly reduced in the ARBs group compared with controls (OR = 0.79; 95%CI: 0.74-0.84). This reduction of T2DM incidence was apparent in the comparison of ARBs to placebo (OR = 0.83; 95%CI: 0.78-0.89), β -blockers (OR = 0.73; 95%CI: 0.62-0.87), CCBs (OR = 0.76; 95%CI: 0.68-0.85) and non-ARBs (OR = 0.57; 95%CI: 0.36-0.91)^[23]. ARBs were associated with significant reduction in the risk of new-onset diabetes in patients with hypertension (OR = 0.74; 95%CI: 0.68-0.81), heart failure (OR = 0.70; 95%CI: 0.50-0.96), impaired glucose tolerance (OR = 0.85; 95%CI: 0.78-0.92) or cardiocerebrovascular diseases (OR = 0.84; 95%CI: 0.72-0.97). A meta-analysis by Abuissa *et al*^[15] of randomized controlled trials associated ARBs treatment with

a reduction of new-onset T2DM [RR = 0.77 (95%CI: 0.71-0.83)]^[15]. Another meta-analysis of randomized clinical trials showed that ARBs had a smaller risk of new-onset T2DM (OR = 0.79; 95%CI: 0.73-0.85; $P < 0.00001$) compared with control groups^[16]. Similarly, Cheung et al in a meta-analysis of studies with ARBs showed that sartans were associated with a decrease of new-onset diabetes^[24].

Telmisartan: Among members of the ARB family, some have the ability to partially activate PPAR γ . Indeed, when various ARBs were evaluated regarding their PPAR γ activating capacity, telmisartan was identified as the most prominent one^[25,26]. Irbesartan was also associated with a milder activation of PPAR γ . However only telmisartan retained its PPAR γ -activating ability in lower concentrations usually attained during oral drug treatment^[25]. This capacity of telmisartan can be attributed, at least partially, to its unique structure which differentiates it from other ARBs as well as to its structural resemblance with pioglitazone, a full PPAR γ agonist^[25]. Telmisartan in contrast to thiazolidinediones is only a partial PPAR γ agonist. This leads to a diverse but overlapping gene expression compared with full activation of PPAR γ and thus bestowing upon telmisartan unique pleiotropic effects^[27].

A number of studies have identified telmisartan as having beneficial effects on glucose homeostasis both in non-diabetic subjects^[28,29] as well as diabetic patients^[30,31]. Furthermore, studies comparing telmisartan with other ARBs have shown that telmisartan had more favorable effects on glycemic profile^[32,33]. Hypertension often co-exists with dyslipidemia as commonly seen in metabolic syndrome. Moreover, there have been studies associating statin treatment with deteriorating effects on glucose metabolism^[34-37]. Indeed, in the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin trial (JUPITER)^[38] rosuvastatin was associated with an increase in physician-reported newly diagnosed diabetes ($P = 0.01$) and an increase in glycated hemoglobin (HbA1c) *vs* placebo ($P = 0.001$). We have shown that telmisartan not only retains its beneficial effects on glucose homeostasis when co-administered with a statin, but also seems to negate any adverse effect of statin therapy on glycemic indices^[39]. Patients ($n = 151$) with mixed dyslipidemia, stage 1 hypertension and prediabetes were randomized to receive rosuvastatin (10 mg/d) plus telmisartan 80 mg/d or irbesartan 300 mg/d or olmesartan 20 mg/d^[39]. After 6 mo, the homeostasis Model Assessment Insulin Resistance (HOMA-IR) index improved only in the telmisartan group (-29%) compared with either irbesartan (+16%; $P < 0.01$ *vs* RT) or olmesartan group (+14%; $P < 0.05$ *vs* RT) ($P < 0.05$ for all *vs* baseline).

A number of large clinical trials have evaluated the effect of telmisartan on the incidence of new-onset T2DM. The Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial (ONTARGET) evaluated the effects of telmisartan on hard clinical endpoints^[40]. High risk patients ($n = 25620$) with

coronary, peripheral or cerebrovascular disease or diabetes with end-organ damage were randomized to 3 groups and were followed for a median period of 56 mo. The first group received telmisartan (80 mg/d), the second group ramipril (10 mg/d) and the third group telmisartan plus ramipril (80/10 mg/d). The ONTARGET trial did not reveal any difference between ramipril (6.7%) and telmisartan (7.5%; HR = 1.12; 95%CI: 0.97-1.29) regarding new onset diabetes^[40].

The Telmisartan Randomised Assessment Study in ACE intolerant subjects with CVD (TRANSCEND) came as a complementary study to ONTARGET^[41]. High risk patients ($n = 5926$) intolerant to ACE-I with coronary, peripheral or cerebrovascular disease or diabetes with end-organ damage were randomized to telmisartan (80 mg/d) or placebo on top of any current therapy. TRANSCEND had the same primary endpoint as ONTARGET. A clear trend in reducing new clinical diagnosis of diabetes with telmisartan was seen in the TRANSCEND trial. The telmisartan group had lower new diabetes incidence (11%) *vs* placebo (12.8%; $P = 0.081$).

The prevention regimen for effectively avoiding second strokes (PROFESS) study evaluated the effects of telmisartan on stroke incidence after a mean period of 30 mo^[42]. Patients ($n = 20332$) with a history of recent ischemic stroke were randomly assigned (2×2) to receive either both aspirin (25 mg/twice daily) and extended-release dipyridamole (200 mg/twice daily) or clopidogrel (75 mg/d); and telmisartan (80 mg/d) or placebo. Similarly, in the PROFESS a trend was seen in reducing new onset diabetes with telmisartan (1.2%) *vs* placebo (1.5%; $P = 0.1$).

Although the TRANSCEND and PROFESS both only showed trends for the reduction of new-onset T2DM, it should be noted that both of them had some limitations regarding their power to identify beneficial effects of telmisartan on diabetes onset. Indeed, more than one third of the TRANSCEND population had already a history of diabetes, thus decreasing the power of the remaining study population to detect any mild beneficial effect on T2DM development. Moreover, a great percentage (37%) of the PROFESS population was already treated with ACE-Is, which have an established overall positive effect regarding new onset diabetes prevention^[43]. Therefore, again any benefits of telmisartan would be harder to detect on-top of an ACE-I therapy. Moreover, the PROFESS had a much smaller follow up period in contrast to studies with ARBs that showed benefits in new onset diabetes like the LIFE^[17] and VALUE^[19]. Indeed, the PROFESS population was monitored for 2.5 years *vs* 4.8 and 4.2 years for the LIFE and VALUE populations, respectively. This difference could explain why telmisartan showed only a trend for reduction of new onset diabetes.

Renin inhibitors

Aliskiren is the first approved renin inhibitor which acts by directly inhibiting the renin enzyme at the point of

RAAS activation, blocking the conversion of angiotensinogen to angiotensin I and decreasing levels of angiotensin I and angiotensin II^[44]. A limited number of studies have evaluated the capacity of aliskiren to affect glucose metabolism. In a recent study, hypertensive patients with abnormal LV diastolic dysfunction but with normal LV systolic function ($n = 78$) were randomized to aliskiren (up to 300 mg/d) treatment or control group which was treated with β -blockers or CCBs^[45]. Fasting insulin and glucose remained unchanged in the aliskiren group, in contrast to the control group where an increase in both fasting insulin ($P = 0.03$) and glucose ($P = 0.003$) were observed. In another double-blind trial, patients with diabetes mellitus and hypertension ($n = 837$) were randomized to once-daily aliskiren (150 mg titrated to 300 mg after four weeks), ramipril (5 mg titrated to 10 mg) or the combination for eight weeks^[46]. No changes in HbA1c and fasting plasma glucose were observed in any treatment group. Another study randomized hypertensive patients with metabolic syndrome to aliskiren (300 mg/d) or losartan (100 mg/d)^[47]. At study end patients performed an euglycemic hyperinsulinemic clamp and insulin sensitivity was assessed by glucose infusion rate. Insulin resistance improved only in the aliskiren group compared with losartan group ($P < 0.05$ between groups).

Mechanisms

The RAAS plays a major role both in the pathogenesis of hypertension as well as glucose homeostasis. As a result, a number of mechanisms have been suggested that can play a role in the overall beneficial effect that drugs which effect RAAS have on glucose metabolism.

Bradykinin may play an important role towards a beneficial effect on glucose homeostasis. The ACE beyond the conversion of angiotensin I to angiotensin II can also decrease bradykinin levels^[48]. Indeed, ACE promotes the degradation of bradykinin to inactive fragments *via* a kininase II - mediated mechanism^[49]. As a result, ACE-I can increase bradykinin levels^[50]. Bradykinin has been shown to promote insulin sensitivity at the skeletal muscle level^[51,52].

The principal glucose transporter protein that mediates insulin-stimulated glucose transport into muscle and adipose tissues is the glucose transporter type 4 (GLUT4), thus playing a key role in the regulation of glucose homeostasis^[53]. Angiotensin II decreases GLUT-4 translocation to the cell membrane^[54,55]. As a result the RAAS inhibition could promote insulin sensitivity. Indeed, the inhibition of AT1 receptors prevented the decline of GLUT-4 in a diabetic rat heart model^[56]. Moreover, both ACEIs and ARBs have been associated with increase of GLUT-4 protein expression in skeletal muscle and myocardium in insulin-resistant animal models^[57].

Moreover, angiotensin II inhibits adipogenic differentiation of human adipocytes *via* the AT1 receptor^[58]. Angiotensin II may inhibit preadipocytes recruitment, resulting in the storage of lipids in muscle and other tissues, thus increasing insulin resistance^[59]. As a result, the

blockade of RAAS would promote the recruitment of preadipocytes thereby increasing the number of small insulin-sensitive adipocytes leading to improved insulin sensitivity.

Furthermore, angiotensin II can promote the production of inflammatory cytokines^[60]. Inflammatory cytokines promote oxidative stress thus also leading to increased insulin resistance. In addition, endothelial dysfunction is also associated with insulin resistance^[61]. Angiotensin converting enzyme inhibitors have also been shown to improve vascular function and insulin-mediated vascular responses^[61]. Furthermore, ACE-I may also have direct beneficial effects on pancreatic β cells^[62].

In addition ACE inhibition can lead to vasodilation of blood vessels^[63]. This vasodilation has as a result the increment of total perfused area and thus increases glucose uptake and insulin sensitivity^[64,65]. The activation of the sympathetic nervous system has also been associated with insulin resistance^[66]. Both ACE-I^[67] as well as ARBs^[68] have been shown to decrease levels of catecholamines such as norepinephrine and epinephrine, thus further contributing to amelioration of insulin resistance.

Potassium levels play a significant role in insulin secretion since hypokalemia substantially impairs the insulin secretory response to glucose. As a result the increase of potassium levels by inhibiting the RAAS may also contribute to the improvement of glucose levels. Moreover, magnesium has also been shown to affect glucose homeostasis. Indeed, magnesium deficiency is associated with both a reduced cellular insulin action^[69] and impaired insulin secretion^[70]. The inhibition of the RAAS system leads to increased magnesium levels. A pooled analysis of studies using ACEIs in patients ($n = 96$) with essential hypertension found that changes in insulin sensitivity index (ISI) were directly correlated to alterations in serum magnesium levels^[71].

CCBS

CCBs are generally considered as having an overall neutral metabolic profile. Indeed, a recent meta-analysis of 10 randomized clinical trials evaluated the effect of CCB treatment on new onset T2DM^[72]. The overall risk of diabetes among subjects taking CCBs was not significant (RR = 0.99; 95%CI: 0.85-1.15). Compared with other classes of antihypertensive drugs, CCBs were associated with a higher incidence of diabetes than ACEIs (pooled risk ratio 1.23; 95%CI: 1.01-1.51) or ARBs (1.27; 95%CI: 1.14-1.42) and a lower incidence compared with β -blockers (RR = 0.83; 95%CI: 0.73-0.94) or diuretics (RR = 0.82; 95%CI: 0.69-0.98).

Another recent meta-analysis of 5 clinical trials compared the efficacy of ARBs and CCBs regarding their effect on insulin resistance as assessed using the HOMA-IR index in non-diabetic patients^[73]. Both ARBs and CCBs had a similar effect on blood pressure reduction. However, ARBs reduced the HOMA-IR index (weighted mean difference -0.65, 95%CI: -0.93--0.38) and fasting plasma insulin (weighted mean difference -2.01, 95%CI:

-3.27--0.74) significantly more than CCBs. A recent re-analysis of data from the NAVIGATOR trial showed that CCBs were not associated with new onset diabetes (HR = 0.95; 95%CI: 0.79-1.13)^[74].

Of note overdose of CCB has been associated with hyperglycemia primarily due to the blockade of pancreatic L-type calcium channels and insulin resistance on the cellular level^[75].

However, not all members of the CCB class have the same effect on glucose homeostasis. Indeed, azelnidipine has been associated with beneficial effect on glucose homeostasis in a diabetic animal model^[76]. Moreover, similar beneficial effects were seen in a small study in non-diabetic patients ($n = 17$) with essential hypertension who had controlled blood pressure levels using amlodipine (5 mg/d)^[77]. Azelnidipine (16 mg/d) or amlodipine (5 mg/d) was administered in a crossover design for 12-wk. Despite similar blood pressure reduction, azelnidipine significantly decreased levels of glucose and insulin 120 min after the 75 g oral glucose tolerance test (OGTT) ($P < 0.05$ *vs* amlodipine). This effect may be associated with the anti-inflammatory effects of azelnidipine^[78], since proinflammatory cytokines have been associated with impaired glucose tolerance^[79]. Furthermore, azelnidipine inhibits the enhancement of sympathetic nervous activity^[80]. Since the activation of the sympathetic nervous system has been associated with insulin resistance^[66], azelnidipine treatment may contribute to the amelioration of insulin resistance.

Another interesting member of the CCB class is manidipine^[81]. A beneficial effect on insulin resistance has been shown with manidipine treatment^[82]. The beneficial effects of manidipine have been observed in both non-diabetic and T2DM patients^[83,84]. Furthermore, we have recently shown that manidipine can ameliorate the possible statin-associated increase in insulin resistance^[85]. In a prospective, randomized, open-label, blinded endpoint study a total of 40 patients with impaired fasting glucose, mixed dyslipidemia, and stage 1 hypertension were included. Patients were randomly allocated to rosuvastatin (10 mg/d) plus olmesartan (20 mg/d) or manidipine (20 mg/d). After 3 mo, a significant increase in HOMA-IR index by 14% ($P = 0.02$ *vs* baseline) was seen in the olmesartan plus rosuvastatin group. On the other hand, HOMA-IR index did not change in the manidipine plus rosuvastatin group ($P = \text{NS}$ *vs* baseline; $P = 0.04$ *vs* olmesartan plus rosuvastatin group). This favorable effect of manidipine may be linked to the drug's capacity to partially activate the PPAR γ which plays a major role in glucose metabolism^[82]. Indeed, the effect of manidipine to activate PPAR γ is about two-thirds of that of pioglitazone, a full PPAR γ activator^[82]. This partial activation of PPAR γ may contribute to the avoidance of side effects commonly associated with thiazolidinediones treatment. Moreover, an increase of adiponectin levels (which are inversely associated with the development of insulin resistance and metabolic syndrome) has been observed with manidipine^[86]. Furthermore, manidipine induces a smaller activation of the sympathetic nervous

system, which can also play a role in the beneficial effects on glucose homeostasis. Indeed, when compared with other CCBs, manidipine is associated with lower levels of plasma norepinephrine^[87].

β -BLOCKERS

A number of studies have associated treatment with β -blockers as having a disadvantageous effect on glucose homeostasis^[88-91]. Indeed, a prospective study of three cohorts, namely the Nurses' Health Study (NHS) I and II and the Health Professionals Follow-up Study evaluated the association between the use of different classes of antihypertensive medications and the risk of T2DM incident^[92]. Treatment with a β -blocker was associated with a greater risk for the development of diabetes. Similarly, in the Atherosclerosis Risk in Communities study β -blockers led to an increase of risk for new-onset T2DM (RR = 1.28; 95%CI: 1.04-1.57)^[4]. A large meta-analysis of patients with hypertension ($n = 94492$) treated with beta blockers evaluated the risk for the development of T2DM^[93]. Beta-blocker therapy resulted in a 22% increased risk for new-onset T2DM (RR = 1.22, 95%CI: 1.12-1.33) compared with non-diuretic antihypertensive agents. On the other hand, a recent reanalysis of data from the NAVIGATOR trial showed that β -blockers were not associated with new onset diabetes (HR = 1.10, 95%CI: 0.92-1.31)^[74].

However, not all members of the β blocker class have similar effect on glucose homeostasis. Indeed, carvedilol as well as nebivolol have shown a differentiation from the rest of the class^[94,95]. The Glycemic Effects in Diabetes Mellitus: Carvedilol-Metoprolol Comparison in Hypertensives (GEMINI) study was a randomized, double-blind, parallel-group trial that compared the effects of carvedilol and metoprolol tartrate on glycemic control^[96]. Patients ($n = 1235$) with hypertension ($> 130/80$ mmHg) and T2DM that were already receiving RAS blockers were randomized to receive carvedilol (6.25-25 mg/twice daily) or metoprolol (50-200 mg/twice daily). Open-label hydrochlorothiazide and a dihydropyridine calcium antagonist were added, if needed, to achieve blood pressure target. While blood pressure control was similar between groups, a difference was seen regarding glucose effects. The HbA1c increased with metoprolol (by 0.15%; $P < 0.001$) but not carvedilol (by 0.02%; $P = 0.65$). Moreover, insulin sensitivity improved with carvedilol (9.1%; $P = 0.004$) but not metoprolol (2.0%; $P = 0.48$ *vs* baseline; $P = 0.004$ between groups). Similarly, a study in subjects with metabolic syndrome compared nebivolol (5 mg/d) with metoprolol (100 mg/d)^[97]. After 12-wk treatment both nebivolol and metoprolol had similarly decreased blood pressure and heart rate. However, metoprolol decreased insulin sensitivity compared with nebivolol ($P = 0.03$).

Mechanisms

Several possible mechanisms that may be responsible

for the disadvantageous effect of β -blockers have been described. Treatment with conventional β -blockers leads to an unopposed α 1-activity which causes vasoconstriction and decreased blood flow to the muscles, which are an important organ in the regulation of glucose homeostasis^[98,99]. As a result a decrease in insulin-stimulated glucose uptake would occur, leading to insulin resistance. Furthermore, β -blockers can also decrease the first phase of insulin secretion from pancreatic β cells^[88,89]. In addition, treatment with β -blockers can also lead to weight gain^[100]. Since increased body weight is strongly associated with insulin resistance^[101], this effect of β -blockers can further deteriorate glucose homeostasis.

DIURETICS

An important class of antihypertensive drugs is diuretics. This class includes loop diuretics such as furosemide, thiazide diuretics such as hydrochlorothiazide, thiazide-like diuretics such as chlorthalidone and potassium-sparing diuretics, such as amiloride, eplerenone and spironolactone.

A number of studies have associated diuretic treatment of hypertension as having a negative effect on glucose homeostasis^[18,102]. Indeed, a meta-analysis of 22 clinical trials with 143153 nondiabetic patients evaluated the effects of various antihypertensive drug classes on diabetes incidence^[43]. Treatment with diuretic was associated with increased risk for new onset diabetes compared with other antihypertensive treatments as well as placebo^[43]. A long-term cohort study with initially untreated hypertensive subjects ($n = 795$) evaluated new-onset diabetes incidence according to antihypertensive treatment^[103]. Diuretic treatment was present in 53.5% of subjects that developed T2DM, compared with 30.4% of patients that did not develop diabetes ($P = 0.002$). Moreover, diuretic treatment was an independent predictor of new onset diabetes ($P = 0.004$). Furthermore, a recent reanalysis of data from the NAVIGATOR trial showed that diuretics were associated with an increased risk of new onset diabetes (HR = 1.23, 95%CI: 1.06-1.44)^[74].

A post hoc subgroup analyses of the ALLHAT study among nondiabetic participants of the study who were randomized to receive chlorthalidone ($n = 8419$), amlodipine ($n = 4958$), or lisinopril ($n = 5034$) evaluated the effects of antihypertensive treatment on glucose levels as well as new-onset diabetes^[104]. Chlorthalidone treatment was associated with a greater risk for developing diabetes compared with the other 2 treatment regimens ($P < 0.001$)^[104]. The Systolic Hypertension in the Elderly Program (SHEP) was a placebo-controlled, double-blind, randomized, multicenter clinical trial that evaluated the efficacy of chlorthalidone in patients ($n = 4736$) with isolated systolic hypertension^[105]. After 3 years of treatment, the incidence of new-onset diabetes was similar between the chlorthalidone (8.6%) and placebo group (7.5%; $P = 0.25$ between groups)^[105]. However, when study participants were re-evaluated after a mean follow-up of 14.3 years, 13.0% of patients developed diabetes in

the chlorthalidone group *vs* 8.7% in the placebo group ($P < 0.0001$)^[106].

Of note, chlorthalidone seems to be differentiated from the rest of the thiazide diuretics class^[107]. Indeed, chlorthalidone has a different chemical structure compared with the rest of thiazide diuretics^[107] as well as the ability to inhibit carbonic anhydrase^[108]. Carbonic anhydrase regulates a number of CV related risk factors^[109,110] and its activity is also directly proportional to increasing blood glucose concentration^[111]. As a result, chlorthalidone may have a more favorable metabolic profile compared with the other thiazide diuretics^[107].

The effects of amiloride on blood glucose levels were evaluated in a study by Stears *et al.*^[112]. Patients with essential hypertension ($n = 37$) received, in random order, 4 wk of once-daily treatment with hydrochlorothiazide (25-50 mg), nebivolol (5-10 mg), combination (hydrochlorothiazide 25-50 mg and nebivolol 5-10 mg), amiloride (10-20 mg), and placebo. Each drug was force titrated at 2 wk and separated by a 4-wk placebo washout. Both amiloride and hydrochlorothiazide had similar changes in blood pressure reduction. However, an increase of glucose levels after a 2 h OGTT was observed with hydrochlorothiazide treatment, while no change was seen with amiloride ($P < 0.0001$).

The Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF) evaluated the effects of eplerenone on new-onset diabetes mellitus in patients ($n = 1846$) with mild heart failure^[113]. After a follow-up of 21 mo, eplerenone had no effect on new-onset diabetes mellitus (HR = 0.94, 95%CI: 0.59-1.52). Another study compared the effects of eplerenone with spironolactone in patients ($n = 107$) with mild chronic heart failure^[114]. Spironolactone increased levels of HbA1c ($P < 0.0001$), while no change was observed in the eplerenone group.

Mechanisms

Among the possible mechanisms through which thiazide diuretics may affect glucose homeostasis, hypokalemia may play an important role^[115]. Indeed, hypokalemia can lead to decreased insulin secretion by β cells in response to glucose, as well as decrease in blood flow in muscles. A quantitative review evaluating studies that used thiazide diuretics, found an inverse relationship between glucose and potassium with thiazide use^[116]. Similar results were observed in an analysis of data from the SHEP study^[117]. In the first year of the study among 3790 nondiabetic participants each 0.5-mEq/L decrease in serum potassium was independently associated with a 45% higher adjusted diabetes risk (95%CI: 24%-70%; $P < 0.001$). However, a prespecified subgroup analysis of metabolic parameter data from patients participating in the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study did not confirm a relationship between hypokalemia and deterioration of serum glucose levels^[118].

Moreover, a decrease in magnesium can be seen with diuretic treatment. This could also contribute to the dis-

advantageous effects of diuretics on glucose homeostasis, since hypomagnesaemia is an independent predictor of T2DM^[119,120]. Furthermore, thiazide treatment is also associated with visceral fat redistribution, liver fat accumulation and low-grade inflammation, which in turn increase the risk of new-onset diabetes^[121].

OTHER ANTIHYPERTENSIVE DRUGS

There is little evidence about the effects of other, less used, antihypertensive drugs on glucose homeostasis. A randomized, double-blind multicenter study compared moxonidine (0.2-0.6 mg/d) with metoprolol (50-150 mg/d) in hypertensive subjects ($n = 127$) with T2DM^[122]. After 12 wk of treatment both groups had similar blood pressure reductions as well as similar HbA1c values. However, fasting plasma glucose decreased in the moxonidine group, while an increase was seen in the metoprolol group ($P < 0.05$). Furthermore, the HOMA-IR decreased with moxonidine in contrast to the increase observed with metoprolol. Another multicenter, prospective, randomized study compared moxonidine with metformin^[123]. Patients older than 40 years old, with impaired glucose tolerance (or diabetes mellitus treated with diet alone) and a body mass index (BMI) of at least 27 kg/m² were treated twice daily with moxonidine 0.2 mg or metformin 500 mg for 16 wk. Compared with metformin, moxonidine decreased the area under the curve for insulin ($P = 0.049$). On the other hand, only metformin significantly decreased fasting plasma glucose ($P < 0.05$ *vs* baseline and *vs* moxonidine) as well as HbA1c ($P < 0.005$ *vs* baseline). Both treatments similarly increased the Matsuda ISI from baseline to a comparable degree ($P < 0.05$ *vs* baseline for both groups). Another randomized open parallel study evaluated the chronic effects of moxonidine *vs* amlodipine in obese hypertensive patients ($n = 40$)^[124]. Plasma levels of insulin 120 min after glucose load, decreased with moxonidine treatment ($P < 0.05$) while no change was seen with amlodipine. A multinational, open-label, observational study, the Moxonidine Efficacy on blood pressure Reduction revealed in a metabolic SYndrom population (MERSY) study evaluated the effects of moxonidine on serum metabolic parameters^[125]. Patients with hypertension received moxonidine (0.2-0.4 mg/d) either as monotherapy or as adjunctive therapy for 6 mo. A beneficial trend in metabolic parameters such as fasting plasma glucose and body weight was observed with moxonidine.

A small study evaluated the effects of doxazosin in hypertensive non-insulin depended diabetic patients^[126]. Doxazosin significantly improved insulin sensitivity during the euglycemic insulin clamp and enhanced OGTT. Similarly another small study showed a beneficial effect of doxazosin (2 mg or 4 mg daily for 3 mo) on insulin resistance indices in hypertensive patients ($n = 21$) with T2DM.

CONCLUSION

Hypertension is associated with increased morbidity and

mortality. Furthermore, hypertensive patients have an increased prevalence of insulin resistance as often is the case with metabolic syndrome subjects. This disturbance in glucose homeostasis further increases the risk for the development of CVD as well as the development of diabetes. The various antihypertensive drug categories have different effects on glucose metabolism. Indeed, ACE-I and ARBs have the most favorable effect on insulin resistance and the development of T2DM. Moreover, CCBs have an overall neutral metabolic effect. However, both azelnidipine and manidipine have been associated with beneficial glucose effects. On the other hand, diuretics as well as β -blockers have been associated with detrimental effects on glucose metabolism.

An interesting query is whether the adverse effects of some antihypertensive drug categories on glucose metabolism and their potency to increase new-onset diabetes mellitus incidence is also associated with an increase in CVD events. It would be reasonable to assume that the drug-induced increases in glucose levels and T2DM incidence would have increased CVD risk similarly to traditional risk factors for new-onset diabetes. However, no such increase in CVD risk was seen in the ALLHAT study in those who developed diabetes in the chlorthalidone treatment arm^[127]. Similarly were the results from the SHEP study^[106]. Diabetes at baseline was associated with increased CV mortality rate (adjusted HR = 1.659, 95%CI: 1.413-1.949) and total mortality rate (adjusted HR = 1.510, 95%CI: 1.347-1.693). Furthermore, diabetes that developed during the trial among subjects on placebo was also associated with increased CV adverse outcome (adjusted HR = 1.562, 95%CI: 1.117-2.184) and total mortality rate (adjusted HR = 1.348, 95%CI: 1.051-1.727). However, diabetes that developed among subjects during diuretic therapy did not have statistically significant associations with CV mortality rate (adjusted HR = 1.043, 95%CI: 0.745-1.459) or total mortality rate (adjusted HR = 1.151, 95%CI: 0.925-1.433). In addition, diuretic treatment in diabetic patients was strongly associated with lower long-term CV mortality rate (adjusted HR = 0.688, 95%CI: 0.526-0.848) and total mortality rate (adjusted HR = 0.805, 95%CI: 0.680-0.952). Of note, even if new-onset T2DM after diuretic or β -blocker is not associated with increased CVD morbidity and mortality, the health care cost should be considered. Indeed, the management and treatment costs of a hypertensive patient with diabetes are far greater compared with a non-diabetic patient.

On the other hand, the Progetto Ipertensione Umbria Monitoraggio Ambulatoriale study, a long-term cohort study in initially untreated hypertensive subjects with a median follow up of 6 years, identified diuretic treatment as an independent predictor of new onset diabetes ($P = 0.004$)^[103]. Of interest, CV event risk was similar between diabetic subjects at study baseline and subjects that developed new-onset T2DM during the study. An interesting study, evaluated hypertensive subjects ($n = 754$) and followed them long term for 25-28 years^[128]. Patients were treated with thiazide diuretics and beta-adrenergic blocking drugs with the addition of hydralazine during

the first decade. Calcium antagonists were substituted for hydralazin and, if needed, ACE-I were added when these drugs became available. After 25 years, treatment with β -blockers was associated with new-onset T2DM. New-onset diabetes was associated with an increased risk for stroke (HR = 1.67; 95%CI: 1.1-2.6; $P < 0.05$), myocardial infarction (OR = 1.66; 95%CI: 1.1-2.5; $P < 0.05$) and mortality (OR = 1.42; 95%CI: 1.1-1.9; $P < 0.05$). The mean observation time from onset of diabetes mellitus to a first stroke was 9.1 years and to a first myocardial infarction 9.3 years.

Despite the various effects of different antihypertensive drugs on glucose homeostasis, the overall expected benefits *vs* the potential risks should always be carefully weighted for each individual patient. As a result, when the benefits for a patient that should receive a treatment with an antihypertensive class with unfavorable glucose profile are greater than the risk of increased insulin resistance, then the glycemic effects of the antihypertensive drug should not disqualify the patient from treatment. Furthermore, there is often some diversity among the members of an antihypertensive class regarding their effect on glucose. As a result, the antihypertensive drug with the least adverse effect on glucose can be selected. Indeed, despite the overall adverse effect of the β -blockers families on glucose homeostasis, newer members of the class, such as carvedilol and nebivolol, have shown that they are clearly different from the rest regarding glucose effects.

Overall, when treating hypertensive patients the physician should carefully assess the individual patient's medical history which often dictates a particular treatment. When there are no contraindications, an antihypertensive drug with a favorable or at least neutral effect on glucose homeostasis should be selected. This way, any beneficial effects of lowering blood pressure would not be shadowed in any way by a worsening of the metabolic profile. Patients with a strong indication for receiving a β -blocker or a diuretic should not be disqualified only because of the negative effect of these drug categories on glucose homeostasis. When a drug with negative effects on glucose homeostasis is selected, the physician should have in mind the possible deterioration of glucose metabolism and increased risk for new-onset diabetes and thus follow-up the patient accordingly.

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WJC 6th Anniversary Special Issues (1): Hypertension

Hypertension and medical expenditure in the Japanese population: Review of prospective studies

Koshi Nakamura, Tomonori Okamura, Katsuyuki Miura, Akira Okayama

Koshi Nakamura, Department of Epidemiology and Public Health, Kanazawa Medical University, Ishikawa 920-0293, Japan
 Tomonori Okamura, Department of Preventive Medicine and Public Health, School of Medicine, Keio University, Tokyo 160-8582, Japan

Katsuyuki Miura, Department of Public Health, and Center for Epidemiologic Research in Asia, Shiga University of Medical Science, Shiga 520-2192, Japan

Akira Okayama, Research Institute of Strategy for Prevention, Tokyo 101-0061, Japan

Author contributions: Nakamura K conceived of this review and drafted the manuscript; Okamura T, Miura K and Okayama A reviewed the manuscript and made the final corrections before submission; all authors read and approved the final version.

Correspondence to: Koshi Nakamura, MD, PhD, Department of Epidemiology and Public Health, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan. knaka@kanazawa-med.ac.jp

Telephone: +81-76-2188093 Fax: +81-76-2863728

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creased further in cases of hypertensive patients who have another concomitant cardiovascular risk factor. In particular, hypertension, especially moderate-to-severe untreated hypertension, increases the risk of long-term hospitalization resulting in considerably higher medical expenditure, compared with non-hospitalized cases. Therefore, assuming that the use of antihypertensive medication is essential for hypertensive patients to prevent serious vascular diseases, a cost-effective high-risk strategy needs to be considered to reduce both ill-health and the economic burden due to hypertension. However, from a population perspective, medical expenditure attributable to hypertension comes mainly from pre-to-mild hypertension. Therefore, there is also a need to consider a population strategy that aims to shift the entire population to lower levels of blood pressure.

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Key words: Hypertension; Medical expenditure; Japan; Cohort study

Abstract

Hypertension is a major determinant of health and is likely to have an effect on medical economics. The economic burden due to hypertension may be attributable not only to antihypertensive medication but also to the very expensive procedures required for cases of cardiovascular disease that occur more frequently in hypertensive compared with normotensive individuals. The objective of this article was to review articles published on prospective cohort studies that measured medical expenditure attributable to hypertension in community-dwelling populations in Japan. Many medical services in these populations are provided under the medical insurance system that requires the enrolment of all Japanese residents. Personal medical expenditure attributable to hypertension increases with worsening severity of the condition. Medical expenditure was in-

Core tip: Hypertension is likely to affect medical economics. We reviewed articles published on prospective cohort studies that measured medical expenditure attributable to hypertension in community-dwelling populations in Japan. Personal medical expenditure attributable to hypertension increased with worsening severity of the condition. Medical expenditure was increased further in hypertensive patients who had another concomitant cardiovascular risk factor. In particular, hypertension, especially moderate-to-severe untreated hypertension, increased the risk of long-term hospitalization. This resulted in considerably higher medical expenditure, compared with non-hospitalized cases. However, from a population perspective, medical expenditure attributable to hypertension is mainly from pre-to-mild hypertension.

Nakamura K, Okamura T, Miura K, Okayama A. Hypertension and medical expenditure in the Japanese population: Review of prospective studies. *World J Cardiol* 2014; 6(7): 531-538 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i7/531.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i7.531>

INTRODUCTION

Hypertension is a major cause of premature death and disability in the world mainly as a result of cardiovascular disease including coronary heart disease and stroke, and other vascular diseases^[1,2]. This burden of ill-health represents an economic burden, which is attributable not only to antihypertensive medication but also to very expensive procedures such as percutaneous coronary intervention, coronary artery bypass grafts, neurosurgical treatment, or hemodialysis that are required in cases of serious vascular diseases that occur more frequently in hypertensive than normotensive individuals. Therefore, only prospective cohort studies can measure medical expenditure attributable solely to hypertension in the general population. This fundamental information is required when considering the cost-effectiveness of treating and preventing hypertension.

Japan provides an ideal situation to measure medical expenditure attributable to hypertension, as it is possible to use epidemiological methods to analyse data on health checkups and medical expenditure. Health checkups are commonly conducted in communities and worksites in Japan, whereas data on medical expenditure are available from the medical insurance system that controls medical cost nationwide and is compulsory for all Japanese residents (see ACKNOWLEDGMENTS)^[3-5]. Several epidemiological studies have used these merits to examine the relationship between hypertension status and medical expenditure after a follow-up period in Japanese populations. The objective of this article is to review articles published on these epidemiological studies in Japan.

SEARCH STRATEGY AND SELECTION

We performed a systematic search on Medline for relevant articles published between January 1966 and January 2014. We searched using medical subject headings (MeSH) terms and text words: {(hypertension [MeSH term], including MeSH terms found below this term in the MeSH tree) or (hypertension [text word]) or (high blood pressure [text word])} and {(costs and cost analysis [MeSH term], including MeSH terms found below this term in the MeSH tree) or (cost [text word]) or (expenditure [text word]) or (expense [text word])} and {(Japan [text word])}. We restricted the search to English language articles so that everyone could read the full texts if necessary. Using this search strategy we identified a total of 163 articles. We set the following inclusion criteria that suited the objectives of our study: (1) prospective cohort, but not cross-sectional studies, that examined the

relationship between hypertension status and subsequent medical expenditure; (2) studies conducted in a general Japanese population, but not a population that consisted solely of individuals with a particular high-risk condition or hospital patients; (3) hypertension status assessed by blood pressure measurement and/or medical history of taking antihypertensive medication, with medical expenditure being measured using insurance claim history files of the Japanese medical insurance system; and (4) studies that provided evidence about how much medical expenditure is incurred by hypertension and/or evidence on any relevant topics. We read the titles and abstracts of all the articles identified in the Medline search to exclude any articles that seemed irrelevant. The full texts of the remaining articles were read to determine if they met our inclusion criteria. Of the 163 articles identified, only six articles were considered as relevant and met our inclusion criteria. Although we manually searched for extra relevant articles in the reference lists of the identified articles and other publications, no additional relevant article was identified from these sources. Of the six relevant articles, three articles were from the same cohort study, but each dealt with different topics without duplicate publication^[6-8]. The remaining three articles were all different^[9-11].

HYPERTENSION AND MEDICAL EXPENDITURE

The first study to report on the relationship between hypertension status and subsequent medical expenditure was the Shiga National Health Insurance (NHI) cohort study^[6]. This study was conducted in seven towns and one village in Shiga prefecture in the central part of Japan, and included 4191 community-dwelling beneficiaries of NHI, an insurance group for self-employed individuals (*e.g.*, farmers and fishermen) and retirees and their dependants. The study participants were aged between 40-69 years and were not taking antihypertensive medication and did not have a history of cardiovascular disease. They were classified into four sex-specific categories according to their blood pressure measured at a baseline survey in 1989-1991. The four blood pressure categories were defined as follows according to the 7th report of the Joint National Committee in the United States^[12]: “normotension” (systolic blood pressure (SBP) < 120 mmHg and diastolic blood pressure (DBP) < 80 mmHg); “prehypertension” (SBP 120-139 mmHg and/or DBP 80-89 mmHg); “stage 1 hypertension” (SBP 140-159 mmHg and/or DBP 90-99 mmHg); and “stage 2 hypertension” (SBP ≥ 160 mmHg and/or DBP ≥ 100 mmHg). The participants were followed up for 10 years from 1990 to calculate the mean medical expenditure per month during the follow-up period. The cumulative hospitalization rate and all-cause mortality for each blood pressure category were also recorded. If a participant withdrew or died, the follow-up period was terminated at that point. The medical expenditure recorded in this study was confined to the fee schedule range used in the medical insurance system

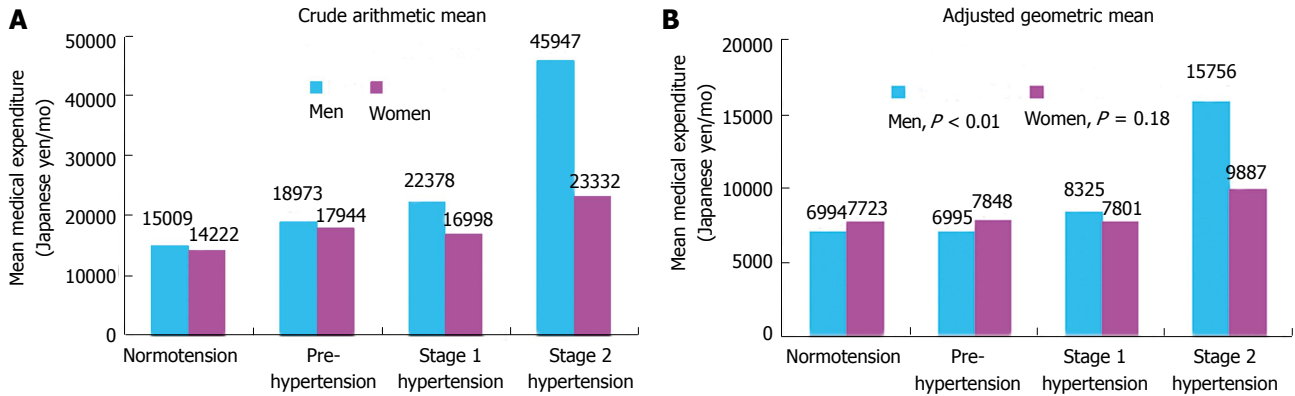


Figure 1 Crude arithmetic mean (A) and adjusted geometric mean (B) of medical expenditure per month over 10 years of follow-up in male and female Japanese medical insurance beneficiaries aged 40-69 years, grouped according to sex and hypertension status. Analysis of covariance was used to compare log-transformed monthly medical expenditure in each blood pressure category, after adjustment for age, body mass index, smoking habit, drinking habit, serum total cholesterol, and a history of diabetes. From Nakamura *et al.*^[6]

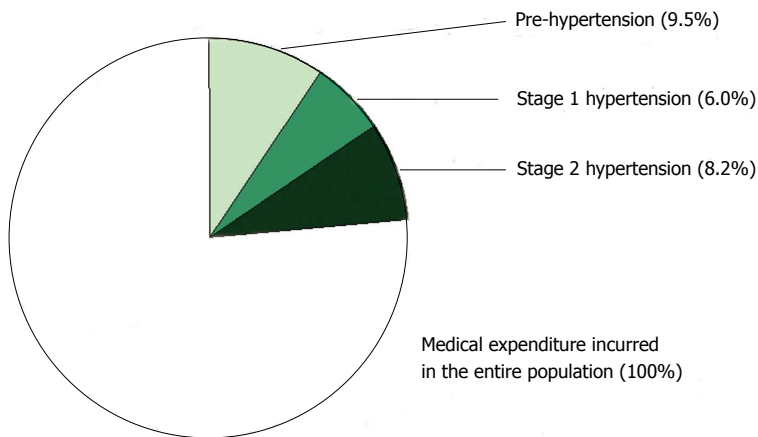


Figure 2 Percentage of medical expenditure attributable to pre-, stage 1, and stage 2 hypertension relative to medical expenditure incurred by the entire population of Japanese medical insurance beneficiaries aged 40-69 years (100%). From Nakamura *et al.*^[6]

in Japan, and was calculated as the sum of expenditure from the insurance organization and the beneficiary. The crude arithmetic mean of medical expenditure increased with worsening severity of hypertension, especially in men (Figure 1A). The adjusted geometric mean of medical expenditure, calculated using analysis of covariance that incorporated logarithmically-transformed values of medical expenditure as the dependent variable and major cardiovascular risk factors as covariates, also correlated positively with blood pressure levels (Figure 1B). The odds ratio for cumulative hospitalization (1.96, 95%CI: 1.29-2.98) and hazard ratio for all-cause mortality (3.19, 95%CI: 1.67-6.08) in stage 2 hypertensive men were also higher than those in normotensive men after adjustment for potential confounding factors. This study estimated medical expenditure attributable to the three grades of hypertension (*i.e.*, “pre-hypertension”, “stage 1 hypertension”, and “stage 2 hypertension”) from a population perspective. The medical expenditure attributable to these three hypertension grades accounted for 23.7% of the medical expenditure incurred in the combined male and female study participants (Figure 2). The percentage for each-hypertension-related medical expenditure was 9.5% for “pre-hypertension”, 6.0% for “stage 1 hypertension”, and 8.2% for “stage 2 hypertension”.

The Ohsaki NHI cohort study^[9] was conducted sub-

sequently in Ohsaki city, Miyagi prefecture in the north-east part of Japan using a similar method. This study included 12340 community-dwelling NHI beneficiaries aged 40-79 years without a history of cardiovascular disease or cancer. The study participants were classified into the following two categories according to their blood pressure and antihypertensive medication status assessed in 1994-1995: “normotension” (SBP < 140 mmHg, DBP < 90 mmHg, and not taking antihypertensive medication); and “hypertension” (SBP ≥ 140 mmHg, DBP ≥ 90 mmHg and/or taking antihypertensive medication). The arithmetic mean of medical expenditure per month during the 6-year follow-up period from 1996 was higher in hypertensive participants than in normotensive participants even after adjustment for age, sex, smoking and alcohol drinking habits, and obesity, hyperglycaemia, and dyslipidemia status: 275.9 United States dollars/mo *vs* 203.5 United States dollars/mo, respectively (1 United States dollar = 115 Japanese yen at the foreign exchange rate given in the article). When the hypertensive participants were divided further into untreated and treated hypertensive subjects, the mean medical expenditure was increased further in the treated hypertensive group than in the untreated hypertensive and normotensive groups: 317.7 United States dollars/mo *vs* 223.0 United States dollars/mo *vs* 202.9 United States dollars/mo, respec-

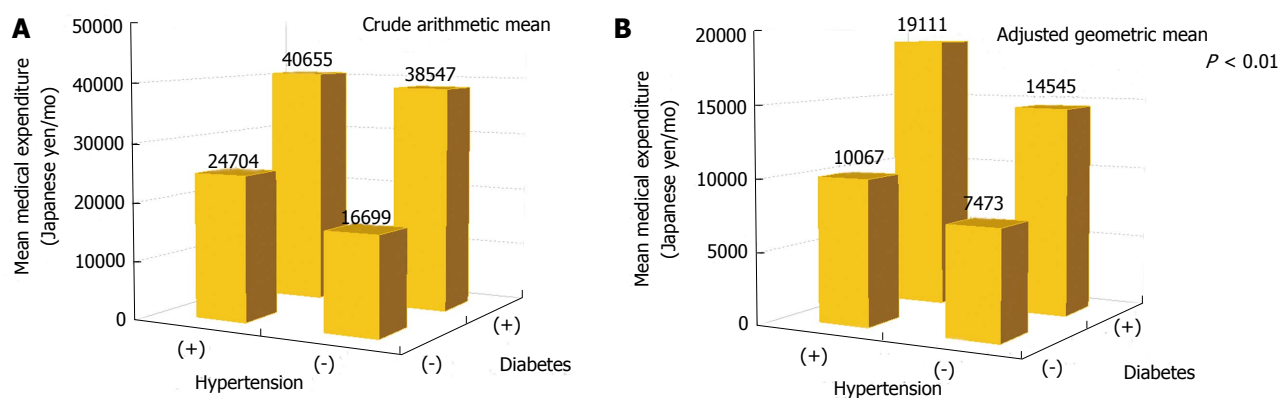


Figure 3 Crude arithmetic mean (A) and adjusted geometric mean (B) of medical expenditure per month over 10 years of follow-up in Japanese medical insurance beneficiaries aged 40-69 years, grouped according to hypertension and diabetes status. Analysis of covariance was used to compare log-transformed monthly medical expenditure in each blood pressure category, after adjustment for age, sex, body mass index, smoking habit, drinking habit, and serum total cholesterol. From Nakamura *et al.*^[7]

tively.

The Ibaraki NHI cohort study^[10], which was conducted over a wide area in Ibaraki prefecture in the eastern part of Japan, used a similar, but partially different method. This study included 42426 community-dwelling NHI beneficiaries aged 40-69 years without a history of cardiovascular disease. The study measured medical expenditure for just one year (2006), four years after the baseline survey in 2002 that assessed hypertension status. Monthly medical expenditure was compared for the same four blood pressure categories as those used in the Shiga NHI cohort study, although stage 2 hypertension included both participants who had a SBP ≥ 160 mmHg and/or DBP ≥ 100 mmHg free from antihypertensive medication and those on antihypertensive medication. The median medical expenditure increased with more severe hypertension in every stratum of age (*i.e.*, 40-54 years and 55-69 years) and sex.

HYPERTENSION COMBINED WITH ANOTHER RISK FACTOR AND MEDICAL EXPENDITURE

The Shiga NHI cohort study^[7] examined the relationship between the combination of hypertension status and diabetes status and subsequent medical expenditure in 4535 participants. This patient group was selected as the coexistence of hypertension and diabetes is often due mainly to insulin resistance accompanied by compensatory hyperinsulinemia^[13,14], which occurs more frequently in obese than in non-obese individuals^[15,16]. The mean of medical expenditure per month over a 10-year follow-up period was compared in the following four categories: “neither hypertension nor diabetes”; “hypertension alone”; “diabetes alone”; and “both hypertension and diabetes”. Hypertension was defined as a SBP ≥ 140 mmHg, a DBP ≥ 90 mmHg, and/or taking antihypertensive medication, while diabetes was defined as having a history of diabetes assessed by a self-reported questionnaire. The participants with both hypertension and

diabetes, who accounted for 1.3% of the study population, incurred on average, higher medical expenditure compared with those without hypertension, diabetes, or their combination, even after adjustment for confounding factors (Figure 3). Similarly, the “both hypertension and diabetes” group had the highest risk of all-cause mortality among the four categories, with an adjusted hazard ratio of 2.21 (95%CI: 1.11-4.42), relative to the “neither hypertension nor diabetes” group.

The Ohsaki NHI cohort study^[9] compared mean medical expenditure per month over a 6-year follow-up period in four similar categories in participants stratified according to the presence or absence of obesity defined as a body mass index ≥ 25.0 kg/m². Hypertension was defined as described earlier, whereas hyperglycemia was defined as a plasma glucose ≥ 150 mg/dL and/or having a self-reported history of diabetes. The results of this study showed a pattern similar to those of the Shiga NHI cohort study for both obese and non-obese participants, although obesity resulted in additional medical expenditure in each of the four blood pressure and plasma glucose categories. In short, non-obese participants with both hypertension and hyperglycemia, with hypertension alone and with hyperglycemia alone had increased medical expenditure of 85.2%, 33.0% and 48.3%, respectively, compared with non-obese participants with neither hypertension nor hyperglycemia. In contrast, obese participants with both hypertension and hyperglycemia had a 91.0% increase in expenditure compared with the same reference group. The medical expenditure attributable to both hypertension and hyperglycemia with and without obesity accounted for 1.4% and 1.8% of the medical expenditure incurred in the entire population, respectively.

The Shiga NHI cohort study^[8] examined the relationship between the combination of hypertension status and smoking status and subsequent medical expenditure in 1708 male participants after excluding male ex-smokers and all females, as smoking is more prevalent in Japanese men than in Japanese women^[17]. Mean medical expenditure per month over a 10-year follow-up period was compared in the following four categories: “neither hyper-

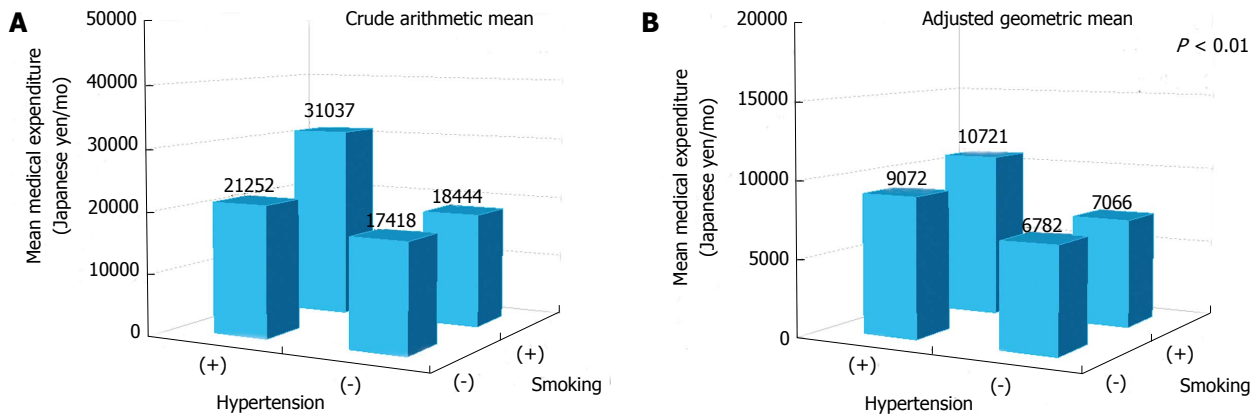


Figure 4 Crude arithmetic mean (A) and adjusted geometric mean (B) of medical expenditure per month over 10 years of follow-up in male Japanese medical insurance beneficiaries aged 40-69 years, grouped according to hypertension and smoking status. Analysis of covariance was used to compare log-transformed monthly medical expenditure in each blood pressure category, after adjustment for age, body mass index, drinking habit, serum total cholesterol, and a history of diabetes. From Nakamura *et al*.^[8]

tension nor smoking”; “hypertension alone”; “smoking alone”; and “both hypertension and smoking”. Hypertension was defined as described earlier, while smoking was defined as currently smoking. Participants with both hypertension and smoking, who accounted for 24.9% of the study population, incurred on average, higher medical expenditure compared with those without hypertension, smoking, or their combination, even after adjustment for confounding factors (Figure 4).

OTHER RELEVANT TOPICS

The latest cohort study collected similar data throughout Japan, and reported interesting results which revealed how hospitalization influenced the causality between hypertension and increased medical expenditure^[11]. Unlike the three previous cohort studies, this study included both NHI beneficiaries (12 local organizations) and beneficiaries in the Employee’s Health Insurance scheme (nine local organizations), which is available for employees and their dependants. Currently, all Japanese people younger than 75 years should be enrolled in either NHI or Employee’s Health Insurance schemes (enrolment ratio, 1:2)^[3]. A total of 314622 participants aged 40-69 years without a history of cardiovascular disease or end-stage renal disease were included in the final analyses. The study participants were age and sex-specifically classified into seven categories according to their blood pressure and antihypertensive medication status assessed at the baseline survey in 2008. The seven blood pressure categories were defined according to the 2007 criteria of the European Society of Hypertension and the European Society of Cardiology^[18]. Participants who were not taking antihypertensive medication were classified into one of the following five categories: “optimal blood pressure” (SBP < 120 mmHg and DBP < 80 mmHg); “normal-to-high normal blood pressure” (SBP 120–139 mmHg and/or DBP 80–89 mmHg); “grade 1 hypertension” (SBP 140–159 mmHg and/or DBP 90–99 mmHg); “grade 2 hypertension” (SBP 160–179 mmHg and/or DBP 100–109 mmHg); and “grade 3 hypertension” (SBP

≥ 180 mmHg and/or DBP ≥ 110 mmHg). The remaining participants, who were taking antihypertensive medication, were classified into one of the following two categories: “well controlled hypertension on treatment” (SBP < 140 mmHg and DBP < 90 mmHg on medication); and “poorly controlled hypertension on treatment” (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg on medication). This study first compared the risk of undergoing hospitalization one year (2009) after the baseline survey in each blood pressure category. In men aged 40-54 or 55-69 years, the risk of undergoing hospitalization in 2009, especially long-term hospitalization, increased with worsening severity of untreated hypertension (bars in Figure 5, results presented only for men and women aged 40-54 years). The “grade 2-to-3 untreated hypertension” group appeared to have a further increased risk of being hospitalized for at least 14 cumulative days than the “well controlled hypertension on treatment” group. The results derived from the female cohorts need to be interpreted with caution because of the lower prevalence of hypertension and the small number of hospitalizations in females compared with males. However, in women aged 40-54 years, the “grade 3 untreated hypertension” group appeared to have a further increase in hospitalization risk compared with the “well controlled hypertension on treatment” group. Participants who were hospitalized, especially long-term, incurred considerably higher medical expenditure compared with non-hospitalized participants, regardless of their hypertension status, age, or sex. Hypertensive participants on medication appeared to incur less than half of the medical expenditure of hospitalized participants, as long as they remained out of hospital for treatment of hypertension alone. However, this study did not clarify whether the use of antihypertensive medication could offset long-term medical expenditure.

The study also compared the risk of incurring extremely high medical expenditure, defined as at least 99th percentile values of the sex-specific distribution of medical expenditure in the year after the baseline survey in each of the blood pressure categories. This comparison was made because of the fact that a very small percent-

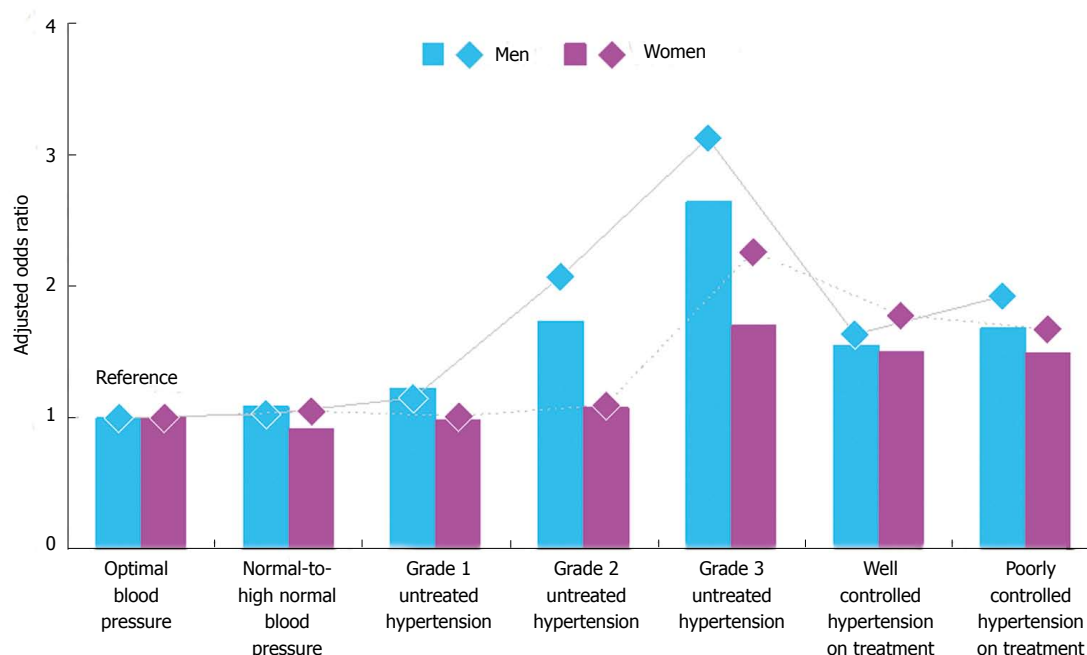


Figure 5 Adjusted odds ratios for two kinds of events over one year of follow-up in male and female Japanese medical insurance beneficiaries aged 40-54 years, grouped according to hypertension status. The bars represent the risk of undergoing hospitalization for ≥ 14 cumulative days, while the diamonds represent the risk of falling into the top 1% group of medical expenditure. A logistic regression model was used to calculate odds ratios after adjustment for age, body mass index, smoking habit, serum low-density lipoprotein cholesterol, log-transformed fasting plasma glucose, and medications for hypercholesterolemia and diabetes, with the “optimal blood pressure” group acting as the reference. Male and female participants who fell into the sex-specific top 1% medical expenditure group each incurred ≥ 1571 euros/mo and ≥ 1249 euros/mo, respectively (1 euro = 95.91 Japanese yen). From Nakamura *et al.*^[11].

age of patients accounts for a substantial percentage of the medical expenditure in the entire population^[19]. Male and female participants who fell into the top 1% group of medical expenditure incurred at least 1571 euros/mo and 1249 euros/mo, respectively (1 euro = 95.91 Japanese yen at the foreign exchange rate given in the article), with the corresponding median cumulative hospitalization periods being 38 and 32 d. The sum of medical expenditure in these top 1% male and female groups accounted for 25.6% and 21.2% of medical expenditure in the entire population, respectively. The risk of incurring such extremely high medical expenditure increased with more severe untreated hypertension in men aged 40-54 or 55-69 years and in women aged 40-54 years (diamonds in Figure 5, results presented only for men and women aged 40-54 years). In men and women aged 40-54 years, the “grade 2-to-3 untreated hypertension” group had a further increased risk of incurring greater medical expenditure compared with the “well controlled hypertension on treatment” group. These results were consistent with the results regarding the risk of being hospitalized for at least 14 cumulative days.

CONCLUSION

Epidemiological studies demonstrated that hypertension caused increased medical expenditure in community-dwelling populations in Japan. Medical expenditure was increased further in hypertensive subjects who had another concomitant cardiovascular risk factor. These studies therefore show that the treatment of hypertension itself is costly. However, attention should be paid to

evidence that hypertension, especially moderate-to-severe untreated hypertension, increased the risk of long-term hospitalization, which resulted in considerably higher medical expenditure compared with non-hospitalized cases. Furthermore, hypertension, especially moderate-to-severe untreated hypertension, increased the risk of surges in medical expenditure, due mainly to long-term hospitalization. Therefore, based on the assumption that use of antihypertensive medication is essential for hypertensive subjects to prevent serious vascular diseases^[20,21], a cost-effective high-risk strategy needs to be considered in order to reduce both ill-health and the economic burden due to hypertension. It should also be noted that from a population perspective, medical expenditure attributable to hypertension appears to result largely from pre-to-mild hypertension, although personal hypertension-related medical expenditure is higher with more severe hypertension. This is in accordance with Rose’s theory that “a large number of people exposed to a small risk may generate many more cases than a small number exposed to high risk”^[22]. Too much focus on hypertensive subjects, especially those with moderate-to-severe hypertension may result in failure to comprehensively reduce the burden of interest. Therefore, there is also a need to consider a population strategy, which aims to shift the entire population to lower levels of blood pressure.

ACKNOWLEDGMENTS

The medical insurance system in Japan

This system requires the enrolment of all Japanese resi-

dents (*i.e.*, “health-insurance-for-all”). Every Japanese resident is able to receive medical services at all clinics and hospitals given approval to provide outpatient medical services and hospitalization. This system consists of three insurance groups (previously two insurance groups) with eligibility for each group depending on the individual’s age and occupation. The fee schedule set by the National Government is uniform across the insurance groups and applies to all the approved clinics and hospitals. Prices are controlled strictly by a fee schedule and are determined on a “fee-for-service” basis. However, recently approximately 20% of acute care hospitals have changed to a flat-fee per day payment system for hospitalized patients according to the diagnosis and procedures undertaken (Diagnosis Procedure Combination/Per-Diem Payment System). The clinic or hospital requests medical expenditure from the insurance organization in which the beneficiary is enrolled and also the beneficiary himself/herself, with the insurance organization paying 70%-90% and the beneficiary paying the balance. However, the medical insurance system does not cover some medical services including health checkups for asymptomatic individuals or inoculations, with annual health checkups available free or at fairly low charges in communities and worksites.

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WJC 6th Anniversary Special Issues (2): Coronary artery disease

Adipose tissue and vascular inflammation in coronary artery disease

Enrica Golia, Giuseppe Limongelli, Francesco Natale, Fabio Fimiani, Valeria Maddaloni, Pina Elvira Russo, Lucia Riegler, Renatomaria Bianchi, Mario Crisci, Gaetano Di Palma, Paolo Golino, Maria Giovanna Russo, Raffaele Calabrò, Paolo Calabrò

Enrica Golia, Giuseppe Limongelli, Francesco Natale, Fabio Fimiani, Valeria Maddaloni, Pina Elvira Russo, Lucia Riegler, Renatomaria Bianchi, Mario Crisci, Gaetano Di Palma, Paolo Golino, Maria Giovanna Russo, Raffaele Calabrò, Paolo Calabrò, Division of Cardiology, Cardio-thoracic and Respiratory Sciences Department, Second University of Naples, AO Dei Colli-Monaldi Hospital, 80131 Naples, Italy

Author contributions: Golia E, Natale F, Fimiani F, Maddaloni V, Russo PE, Riegler L, Bianchi R, Crisci M, Di Palma G collected the data and reviewed literature; Golia E, Limongelli G and Calabrò P wrote the paper; Golino P, Russo MG, Calabrò R and Calabrò P reviewed and approved the manuscript.

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Correspondence to: Paolo Calabrò, MD, PhD, Division of Cardiology, Cardio-thoracic and Respiratory Sciences Department, Second University of Naples, AO Dei Colli-Monaldi Hospital, via Bianchi, 80131 Naples, Italy. paolo.calabro@unina2.it

Telephone: +39-08-17062815 Fax: +39-08-17064234

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response, which characterizes obesity and metabolic syndrome. This might represent an important pathophysiological link with atherosclerotic complications and cardiovascular events. A great number of adipocytokines have been described recently, linking inflammatory milieu and vascular pathology. The understanding of these pathways is crucial not only from a pathophysiological point of view, but also to a better cardiovascular disease risk stratification and to the identification of possible therapeutic targets. The aim of this paper is to review the role of Adipocytokines as a possible link between obesity and vascular disease.

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Key words: Adipocytokines; Obesity; Metabolic syndrome; Coronary artery disease; Inflammation

Core tip: Our article, provide a comprehensive review of the evidence about adipose tissue and cardiovascular risk, focusing on the pathophysiological and clinical role of fat-derived mediators, the so-called adipocytokines.

Abstract

Obesity has become an important public health issue in Western and developing countries, with well known metabolic and cardiovascular complications. In the last decades, evidence have been growing about the active role of adipose tissue as an endocrine organ in determining these pathological consequences. As a consequence of the expansion of fat depots, in obese subjects, adipose tissue cells develop a phenotypic modification, which turns into a change of the secretory output. Adipocytokines produced by both adipocytes and adipose stromal cells are involved in the modulation of glucose and lipid handling, vascular biology and, moreover, participate to the systemic inflammatory

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INTRODUCTION

Obesity is rapidly spreading to epidemic levels in Western and developing countries, becoming a serious health issue. It is associated with increasing morbidity and mortal-

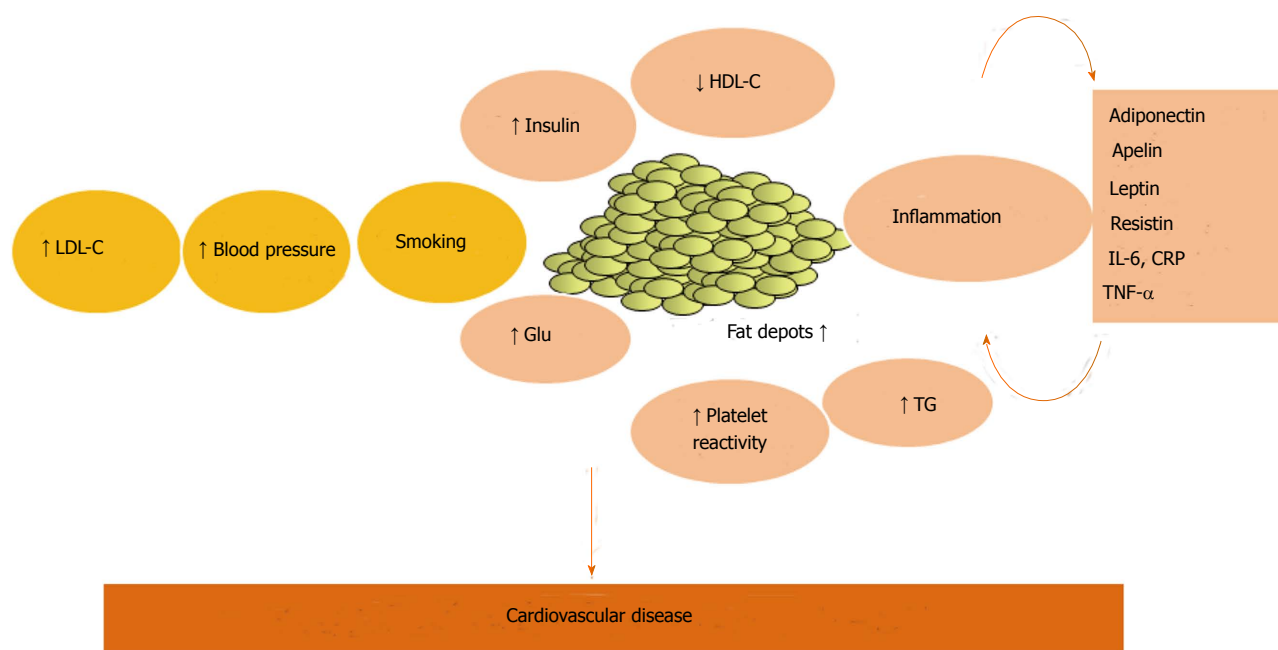


Figure 1 A cartoon illustrating the complex interplay between traditional and non-traditional risk factors in the pathophysiological continuum which leads to cardiovascular disease, with the emerging role of inflammatory pathways. LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; CRP: C-reactive protein; TG: Triglycerides; Glu: Glucose; IL-6: Interleukin-6; TNF: Tumor necrosis factor.

ity^[1]. Obesity shares several features with metabolic syndrome (MetS) and both are associated with well known risk factors for cardiovascular disease (CVD)^[2], such as glucose and lipid metabolism impairment, endothelial dysfunction and atherosclerosis, finally leading an increased risk of cardiovascular events^[2].

Recent evidence claimed that inflammation might represent the pathophysiological link between obesity and MetS (Figure 1), and increasing interest has been developed in the role of adipose tissue as an active trigger of this systemic inflammatory response^[3].

Since adipose tissue is capable of releasing several mediators, the so-called adipocytokines, it is now considered an endocrine organ, affecting metabolism and vascular function. The understanding of these pathways is crucial not only from a pathophysiological point of view, but also to a better cardiovascular disease (CVD) risk stratification and to the identification of possible therapeutic targets.

ADIPOSYTY AND CARDIOVASCULAR RISK

There is consistent epidemiologic evidence for an independent association between obesity and CVD^[4]. In several large, prospective, long-term studies, obesity was independently associated with all-cause mortality and death from CVD in both women and men. The Nurses' Health Study evaluated the relationship between body mass index (BMI) and mortality in 115195 women^[5]. A significant trend for increasing risk of death with increasing BMI and an association between BMI and cardiovascular mortality were found.

In the Framingham Heart Study, obesity was an in-

dependent risk factor for all-cause mortality among male participants, followed up for 30 years^[6]. However, recent data have demonstrated an "obesity paradox", with obese patients suffering from CVD showing a better short- and long-term prognosis than leaner matched subjects^[7]. In particular, in a large meta-analysis^[8], the overall obesity population, defined by BMI, showed an increased risk of mortality compared with normal BMI. However, overweight patients (BMI 25-30 kg/m²) had a significantly 6% lower mortality than patients with normal BMI. This finding was more pronounced in older cohorts^[9]. Interestingly, several studies^[7] suggested an influence of body fitness and on the relationship between adiposity and CVD prognosis. Thus obesity paradox seemed evident in patients with low fitness.

Adipose tissue depots

The so-called visceral obesity is now well-known to be associated to a higher mortality than the peripheral obesity, since it is linked to a higher prevalence of dysmetabolism and hypertension and endothelial dysfunction^[3]. This evidence highlighted the importance of adipose tissue location in determining its unfavourable effects.

Obesity is characterized by an expanded adipose tissue mass^[10] and, interestingly, as noted above, in overweight subjects a typical pattern of adipokines is present, which negatively affect metabolic situation and cardiovascular function^[11].

Typically, the source of these substances are organs (the liver), and immune cells^[12-14]. Most notably, several studies indicate that fat could either regulate the synthesis of these molecules or could be itself an immediate source.

The complexity of adipose tissue as an endocrine organ has been highlighted in several studies which have reported important differences among adipocytes from different depots. These characteristics may account for a differential contribution to obesity-related disorders^[15].

Adipocytes from brown adipose tissue (BAT) are mainly represented in fetuses and newborn and are implicated in thermogenesis^[16,17]. A small amount of BAT persists in adult human AT^[17].

On the other hand, white adipose tissue (WAT) represents a main kind of adipose tissue in adults^[15], largely present in the subcutaneous region (SAT) or close and within the abdominal viscera (VAT).

Vague^[18] first described a link between increased VAT and atherosclerosis, diabetes, and other diseases. This association might be explained by the increased production of mediators, acting with endocrine, autocrine and paracrine mechanisms^[3]. Bigger VAT adipocytes in obese subjects are related to a higher mediators release, comparing to SAT^[19,20]. These factors, adipocytokines include molecules like tumor necrosis factor- α (TNF- α), leptin, adiponectin, resistin, plasminogen activator inhibitor-1 (PAI-1), apelin, interleukin-6 (IL-6), resistin, angiotensinogen, serum amyloid A (SAA), and C-reactive protein (CRP)^[21].

Moreover, several inflammatory cells^[19] are largely represented in the WAT stroma. These cells play an important role in tissue homeostasis, such as the clearance of necrotic adipocytes^[18], increased in obesity. In particular, macrophages produce the majority of TNF- α and increase IL-6 and inducible-Nitric Oxide synthase expression. Then they were thought to be the primary source of the cytokines in adipose tissue^[19,22,23]. However, we have demonstrated with an *in vitro* model, that the mature adipocytes fraction isolated from human adipose tissue is directly involved in both CRP and SAA release^[24].

Interestingly, among visceral fat depots, another specific local fat depot has been studied in the last decade for its relationship with MetS and coronary artery disease (CAD), *i.e.*, epicardial adipose tissue (EAT). EAT surrounds the heart, within the pericardium, it is in contact with coronary vessels (*i.e.*, perivascular) and the surface of ventricles. It shows a higher rate of lipolysis and lipogenesis comparing to other fat depots^[25]. EAT is involved in myocardial energy supply, thermoregulation, and protection of the cardiac autonomic nervous system as well as in the regulation of coronary vessel motion and lumen diameter^[26].

In post-mortem series, epicardial fat have been reported to account for 20% of total heart weight^[27]. Obesity is associated with an increase in the amount of EAT. Both total weight of epicardial fat and epicardial fat cells size correlate with body weight^[28]. Moreover, epicardial fat assessed by echocardiography correlated significantly in multivariable analysis with key components of the MetS, including waist circumference, which is known to parallel the increase in VAT seen in MetS^[29,30]. Then, the cardioprotective features of EAT might be somewhat blunted by the increase in cardiovascular risk carried by its pathological enlargement in obesity^[31].

From a clinical point of view, several clinical and epidemiological studies have found an association between EAT and cardiometabolic risk factors and early stages of atherogenesis. The echocardiographic EAT evaluation was found to be associated with arterial stiffness and intima-media thickness (IMT), two indexes of subclinical atherosclerosis, in hypertensive patients in a study from our group^[32]. Among the 459 patients examined, subjects with epicardial fat > 7 mm were older, had higher systolic, diastolic, and pulse pressure, increased left ventricular mass index, carotid IMT, diastolic and stiffness parameters compared with those with epicardial fat < 7 mm. Age, carotid IMT, and stiffness parameters were independently related to epicardial fat. These findings have been confirmed in a more recent study by Choi *et al.*^[33].

Importantly, the Framingham Heart Study^[34] and Multi-Ethnic Study of Atherosclerosis^[35] identified EAT volume as independent risk markers for CVD. Moreover, recently results of the Heinz Nixdorf Recall study, a population-based prospective cohort study, have been published^[36]. Among the 4093 participants incidence of coronary events increased by quartile of EAT. Doubling of the EAT, measured by computed tomography (CT) scan, carried a 1.5-fold risk of coronary events after adjusting for cardiovascular risk factors and coronary artery calcium score.

Comparing to subcutaneous fat, EAT shows a more dense inflammatory cell infiltrate, predominantly represented by macrophages^[37], and it produces highly atherogenic and inflammatory adipocytokines in patients with CAD^[38].

EAT might then participate also in the inflammatory process within the atherosclerotic plaque. The incidence and severity of CAD and coronary calcification have been in fact associated with EAT thickness and volume^[39].

A recent study assessed the relationship between EAT volume and plaque vulnerability in significant coronary stenosis using intravascular ultrasound. Authors found a positive correlation between EAT volume, measured by CT scan and the percentage of necrotic plaque tissue, and an inverse correlation between with the percentage of fibrous tissue^[40]. Low-density lipoprotein (LDL) cholesterol level and EAT volume were independently associated with the percentage of necrotic plaque tissue. These findings are consistent with previous reports^[41].

Moreover, EAT is associated also with microvascular dysfunction in the absence of obstructive CAD. In a recent study, patients underwent Rb-82 positron emission tomography to obtain standard myocardial perfusion index and myocardial flow reserve (MFR). EAT thickness, EAT volume and coronary calcium score values were higher in patients with impaired flow reserve, with EAT thickness showing the strongest negative correlation with MFR^[42].

OBESITY, VASCULAR INFLAMMATION AND ATHEROSCLEROSIS

In last decades, it became clear that atherosclerosis is

Table 1 Possible link of resistin, C-reactive protein and apelin with vascular inflammation, atherosclerosis and cardiovascular disease

Adipokine	Modulation of inflammation	Association with CVD
Leptin	↑ T cell activation and Th lymphocyte response ↑ cytokine release ↑ NK cell activation ↑ macrophages' cytokine release Activates neutrophils ↑ chemotaxis ↑ oxidative stress ↑ CRP production from endothelium	↑ blood pressure ↑ atherothrombosis: ↑ cholesterol accumulation in vessel wall ↑ adhesion molecules (ICAM, VCAM) expression ↑ endothelial dysfunction (increasing eNOS production, ↓ NO, ↑ET-1) ↑ proliferation and migration of EC and VSMC ↑ ROS accumulation ↑ VSMC apoptosis ↓ angiogenesis ↑ platelet activity ↓ fibrinolysis ↑ PAI-1 ↑TF release from mononuclear cells Induce insulin resistance Associated with HDL-C and inversely with LDL-C ↓ atherothrombosis: ↓ ICAM-1, VCAM-1, and E-selectin ↓ Transformation of macrophages to foam cells ↓ vascular muscular smooth cells proliferation and migration ↑ TIMP, through the increase in IL-10 ↑ oxidation of free fatty acids in several tissue ↑ insulin sensitivity
Adiponectin	↓ T cell activation and proliferation ↓ NF-κB Increases IL-10 Inhibits CRP and IL-6 release	Associated with HDL-C and inversely with LDL-C ↓ atherothrombosis: ↓ ICAM-1, VCAM-1, and E-selectin ↓ Transformation of macrophages to foam cells ↓ vascular muscular smooth cells proliferation and migration ↑ TIMP, through the increase in IL-10 ↑ oxidation of free fatty acids in several tissue ↑ insulin sensitivity

CVD: Cardiovascular disease; CRP: C-reactive protein; EC: Endothelial cells; ET-1: Endothelin-1; ICAM: Intercellular adhesion molecule; HDL-C: High density lipoprotein cholesterol; IL: Interleukin; LDL-C: Low density lipoprotein cholesterol; NF-κB: Nuclear factor κB; NK: Natural killer; NO: Nitric oxide; PAI-1: Plasminogen activator inhibitor-1; TF: Tissue factor; TIMP: Tissue inhibitor of metalloproteinase; VCAM: Vascular cell adhesion molecule; VSMC: Vascular smooth muscle cells.

other than a cholesterol storage disease. A large body of literature highlighted the possible role of inflammation as causal factor in atherogenesis, from endothelial dysfunction to clinical events^[43]. One of the first recognized stages of the atherosclerotic process consists of LDL intimal deposition and endothelial dysfunction. This is caused by the imbalance between nitric oxide (NO) and prostacyclin (PGI₂)-mediated vasorelaxation and the increase in endogenous vasoconstrictors, such as endothelin-1 (ET-1)^[44]. LDL become then oxidized (ox-LDL) by local reactive oxygen species (ROS) and subsequently induce endothelial cells to express adhesion molecules, such as vascular cell adhesion molecule-1, intercellular adhesion molecule 1, and selectins^[45]. This together with the secretion of chemoattractant mediators, such as complement factors, IL-8, monocyte chemoattractant protein-1, determines mononuclear cells recruitment into the vascular wall. Thus, macrophages recruited to intima become “foam cells”, *via* ox-LDL phagocytosis^[44]. Subsequent stages include the transition of the atherosclerotic plaque from the “fatty streak” to a more fibrous lesion. Main actors in this stage are vascular smooth muscle cells, which accumulate in the intima and produce extracellular matrix (ECM)^[44].

Inflammation plays a key role in plaque destabilization and rupture which cause acute vascular events. High rate of vascular inflammation interferes with fibrous cap formation, induces apoptosis and degradation of the ECM, *via* an upregulation of metalloproteinase. The subsequent activation of the coagulation cascade leads to intravascular thrombus formation and the acute clinical events. In this setting, tissue factor (TF) plays a pivotal role in the

pathophysiology of acute coronary syndromes by triggering the formation of intracoronary thrombi following endothelial injury^[45].

As noted above, from a pathophysiological point of view, adipose tissue is not an innocent bystander in this process since it is found to be capable of producing enzyme, cytokines, growth factors and hormones which might affect each one of the stages described above. Moreover, even if the majority of patients suffering of cardiovascular diseases had at least one traditional risk factor^[46,47], almost 25% of subjects did not show any of those^[48]. In this setting, identifying new risk factors might increase our ability to discover and take care of high-risk patients. Within novel prediction factors, adipocytokines have been studied.

Leptin

Leptin has been the first adipocytokine identified as the product of the *ob* gene in obese *ob/ob* mice^[49], which participate in the signalling of fat stores^[50].

Then, early studies on leptin focused on its role in obesity and potential therapies to control it. However, soon it became evident a broader biological role for leptin, including its potential implication in leading to cardiovascular complications in obese patients (Table 1). However, conflicting results on leptin role in CVD have been reported.

In animal and *in vitro* models, leptin promotes atherogenesis, through an increase in oxidative stress in endothelial cells^[51], an increased platelet reactivity and thrombosis^[52]. On the other hand, several studies reported opposite effects such as induction of nitric oxide

production, with anti atherogenic properties^[53].

Most *in vivo* studies in humans demonstrated that leptin levels are linked with cardiovascular risk factors, like hyperlipidemia or hypertension^[54-57], and markers of endothelial dysfunction, thrombophilia and inflammation. Finally, several prospective trials described a link of leptin levels with atherosclerosis and myocardial infarction (MI)^[58-61].

In the West of Scotland Coronary Prevention Study, including 377 male participants and 738 controls^[59], a 20% increase in the incidence of CAD was associated with each standard deviation increase in leptin levels, even after adjustment for potential confounders. Sattar *et al*^[54] found a moderate association, although non significant, between leptin levels and CAD risk. This association was attenuated by BMI, but continued to be significant with other validated risk factors, including several markers of inflammation. Also in female population no significant link between leptin plasma levels and CAD was noted^[61]. On the other hand, Kappelle *et al*^[62], in healthy men, demonstrated a positive association of leptinemia and leptin/adiponectin ratio with incident CVD, even after additional adjustment for several potential confounders, such as clinical risk factors, lipid and microalbuminuria.

In patients with known CAD, leptin might predict future cardiovascular events independent of other risk factors, including lipid status and CRP, according to several reports. Wolk *et al*^[60] followed up 504 patients who had undergone coronary angiography for both stable angina (SA) and ACS for up to 4 years. In the field of ACS, admission plasma leptin levels is associated with the success of thrombolytic therapy in patients with STEMI presenting < 6 h^[63]. In particular, authors found higher rates of thrombolysis failure in patients with basal plasma leptin levels ≥ 14 ng/mL, in comparison with patients with levels less than 14 ng/mL.

The association between plasma leptin and adiponectin levels and recurrent cardiovascular events after an ACS has been investigated in The Long-Term Intervention with Pravastatin in Ischaemic Disease study^[64]. Leptin was a significant and independent predictor of recurrent cardiovascular events.

Consistent with this report, Rajendran *et al*^[65] measured leptin levels, high-sensitivity C Reactive protein (hs-CRP) and IL-6 levels in patients with acute myocardial infarction (AMI), showing higher levels of leptin than control subjects.

The relationship between serial serum leptin levels measurement after thrombolysis for AMI and the degree of coronary atherosclerosis, coronary reperfusion, echocardiographic findings, and clinical outcome was investigated by Khafaji *et al*^[66] in a small study. Leptin concentrations peaked 36 h after admission. Significant correlation of mean serum leptin with reduced ejection fraction and a trend for an increase in the mean serum leptin levels with increasing number of diseased vessels were found. However, there was no correlation between serum leptin levels and outcome or myocardial reperfusion.

Karakas *et al*^[67] in a population-based case-cohort

study within the MONICA/KORA studies. After adjustment for various confounding factors, neither increased leptin levels or low adiponectin were associated with the incidence of coronary events in healthy subjects. Moreover, the leptin/adiponectin ratio didn't improve the ability of the single adipocytokine to predict incident CAD. In another study conducted among patients with ACS and controls, lipid profiles, leptin, pregnancy associated plasma protein A (PAPP-A) and CRP levels were assessed as markers of plaque vulnerability^[68]. Significantly higher levels of leptin, PAPP-A and high-sensitivity CRP (hs-CRP) were observed in cases. At the multivariable analysis, leptin was not independently associated with ACS, while a positive correlation between CRP and leptin concentrations was noted.

Higher adiponectin and lower leptin levels were found to be associated with a high incidence of adverse events also in a Japanese cohort after successful emergency percutaneous coronary intervention for AMI^[69]. A low leptin to adiponectin ratio remained a significant independent predictor of adverse events during long-term follow-up at the multivariable analysis.

Similar observations came earlier from Ku *et al*^[70]. They found that subjects with low baseline leptin levels had higher subsequent CV events and death. Interestingly, although subjects with low leptin had fewer co-morbidities and more favorable metabolic and inflammatory profiles, they showed a worse prognosis than subjects with higher leptin levels. This could be an example of "reverse epidemiology"^[71,72], whereby a predictor of disease becomes inversely associated with prognosis once the disease has developed. According with this idea, a second paper described an association between low leptin levels and cardiovascular death in patients with chronic kidney disease^[73]. Moreover, Leptin is elevated in chronic CAD. Multiple reports have shown that leptin causes coronary vasodilatation, activates endothelial progenitor cells, prevents lipid accumulation, and protects against ischemia-reperfusion injury^[53,60,74,75]. Then a relative leptin deficiency might explain poorer prognosis seen in subjects with established CAD. Finally, the lack of association between leptin and mortality, especially in patients with higher BMI, could be otherwise explained by leptin resistance^[76].

Adiponectin

Adiponectin is a well-described adipocytokine, traditionally reported as a protective factor with an anti inflammatory effect (Table 1)^[11]. It is clear that its circulating levels decrease with weight gain and are inversely correlated to the amount of VAT, as illustrated in CT scan studies^[11]. Interestingly, decreased adiponectin was associated with enhanced pro-inflammatory phenotype in EAT in patients with CAD^[77]. As noted above about leptin, growing conflicting data on adiponectin levels are emerging, suggesting higher complexity of its role, than previously thought. This is particularly evident in the balance between obesity, cardiovascular effects, and prognosis.

Consistent with a putative protective role, Ouchi *et al*^[78]

first detected lower adiponectin levels in subjects with established CAD. Following this experience, several cross-sectional and prospective studies have confirmed an inverse correlation between plasma adiponectin levels and incidence, severity and outcome of CAD^[79-82].

Recent studies, however, failed to demonstrate this correlation^[83,84], or even showed a paradoxical link between higher adiponectin levels and negative events, especially in patients with known CAD or at high cardiovascular risk^[85]. Moreover, Zhang *et al.*^[86] demonstrated higher adiponectin levels in patients with stable CAD and inducible ischemia.

In the Pravastatin Or atorvastatin Evaluation and Infection Trial-Thrombolysis in Myocardial Infarction 22^[87], plasma adiponectin was measured in 3931 subjects with Acute Coronary Syndrome. Adiponectin was negatively associated with age, diabetes, BMI, and triglycerides, while a positive link was noted with the risk of death, MI, and heart failure.

Seven hundred and thirty-five consecutive patients with STEMI treated with primary percutaneous coronary intervention were included in a report by Lindberg *et al.*^[88]. Plasma adiponectin was measured immediately before the procedure. Patients with highest adiponectin quartile had increased mortality compared to patients with low adiponectin. After adjustment for conventional risk factors high adiponectin concentration remained an independent predictor of all-cause mortality.

Also in a cohort from the Jackson Heart Study, conducted among African Americans, Adiponectin was associated with a higher risk of incident stroke in women^[89].

This conflicting evidence has highlighted the complex role of adiponectin in the pathogenesis of CVD. Interestingly, the paradoxical association of adiponectin with a worse outcome was found in a population with a more advanced disease status, which might trigger a compensatory increase in adiponectin levels^[71]. Otherwise, the worse long-term outcome could be the result of the more advanced disease status *per se*. Another possible explanation of these findings might be a condition of relative resistance to adiponectin, as suggested by animal models^[85].

In two recent meta-analysis of prospective studies, it has been shown that plasma adiponectin levels are not related to the risk of CAD or stroke in apparently healthy^[90] and diabetic patients^[91]. Another reason why this association could not be found might lie in adiponectin isoforms. In particular, several studies now consider the high molecular weight (HMW) form to be biologically active^[92]. However, only few cohort studies have prospectively evaluated the association of HMW adiponectin with CAD^[92-94].

Among 30111 women from the Nurses' Health Study, high levels of total and HMW adiponectin, and HMW/total adiponectin ratio were associated with a lower risk of CAD. These associations were largely mediated by parameters related to glucose and lipid metabolism and inflammation, especially HDL-cholesterol levels^[95]. In a study by Kunita *et al.*^[96], in 394 patients referred for com-

puted tomography angiography (CTA), levels of plasma HMW adiponectin were evaluated. In patients with obstructive CAD HMW adiponectin was significantly lower than that in patients without. Furthermore, it was significantly associated with the disease extent and with characteristics of plaque instability, such as positive remodeling, low CT density and adjacent spotty calcium. In a recent nested case-control study conducted among 15566 free of CVD subjects, baseline total and HMW adiponectin and their ratio were examined^[97]. After adjustment for matched variables and traditional risk factors, total and HMW adiponectin and their ratio were not associated with overall risk of CVD. However, the highest quartile for HMW adiponectin and HMW/total adiponectin ratio decreased risk of CVD compared with the lowest quartile among middle-aged individuals with high blood glucose, while this association was not seen among the elderly.

Resistin

Resistin was discovered as a fat-derived molecule in obese mice, for its capacity of inducing insulin resistance^[98,99]. The main source in animals were adipocytes, in particular, from abdominal depots^[98]. The substantial lack of homology between human and mouse resistin genes made difficult to confirm these observation in humans. In particular, in humans resistin is produced mainly by stromal vascular cells, rather than adipocytes^[100]. However, resistin expression has been reported in human WAT by preadipocytes^[101]. Even the relationship between resistin and insulin resistance, overweight and DM type 2 was extensively reported in human with non consistent conclusions^[99,102-104].

Considering that macrophages are its main source in humans, a main pro-inflammatory role has been hypothesized for Resistin. Thus, several *in vitro* studies was conducted (Table 2), which illustrated that Resistin levels increased in response to endotoxin and proinflammatory cytokines administration^[104]. Moreover, Chen *et al.*^[105] recently reported that ox-LDL induced resistin mRNA expression in cultured adipocytes. In a recent study from our group^[106], resistin induced prothrombotic phenotype of human coronary artery endothelial cells (HCAECs). HCAECs incubated with resistin showed an upregulation of TF expression, and its activity was induced in a dose-dependent manner through the activation of NF-κB pathway.

In light of these evidences, resistin has been studied for its implications in CAD.

In apparently healthy individuals from the European Investigation into Cancer and Nutrition-Potsdam Study, individuals in the highest quartile of resistin levels, compared with the lowest quartile, had a relevant increased risk of myocardial infarction but not of ischemic stroke^[107]. This association persisted even after adjustment for CRP levels. In contrast, subsequent study described the independent link between resistin and the incidence of ischemic stroke within post menopausal women^[108].

In a recent study of 6636 adults recruited from general population, after 3.5 years of follow-up, the group in

Table 2 Possible link of resistin, C-reactive protein and apelin with vascular inflammation, atherosclerosis and cardiovascular disease

Adipokine	Modulation of inflammation	Association with CVD
Resistin	↑NFκB dependent cytokine release and adhesion molecule expression (including TNF-α/IL-6) on endothelial cells ↑ proliferation of vascular smooth muscle cells through ERK and PI3K pathways	Endothelial dysfunction: ↓ NO and EDHF ↑ ET-1 release ↑ VEGF and MMP ↑ expression of adhesion molecules and chemokines ↓ TRAF-3 Controversial effects in humans on insulin resistance and type 2 diabetes
CRP	↑ expression of ICAM, VCAM, E selectin recruitment of mononuclear cells through MCP 1 VSMC proliferation, migration ↑ CD4 ⁺ Lymphocytes ↑ gamma - INF production ↑ ET-1 release ↑ CD40/CD40L on endothelium ↑ complement activation	↑ endothelial dysfunction through ↓ NO vasodilatation ↑ oxidized LDL opsonization and macrophages uptake with subsequent foam cells formation ↑ in vessel wall oxidative stress ↑ TF and PAI 1 ↑ MMP
Apelin	↓ superoxide radicals ↓ NADPHO ↓ oxidative injury ↑ NO ↑ vascular progenitor cells mobilization	↑ inotropism ↑ neoangiogenesis ↓ endothelial dysfunction ↓ Ang II and BP ↓ myocardial damage after infarction ↑ cholesterol efflux from macrophages

Ang: Angiotensin; BP: Blood pressure; CRP: C-reactive protein; CVD: Cardiovascular disease; EDHF: Endothelium-derived hyperpolarizing factor; ERK: Extracellular signal-regulated kinase; ET-1: Endothelin-1; HDL-C: High density lipoprotein cholesterol; ICAM: Intercellular adhesion molecule; IL: Interleukin; INF: Interferon; LDL-C: Low density lipoprotein cholesterol; MCP: Monocyte chemoattractant protein; MMP: Metalloproteinase; NADPHO: Nicotinamide adenosine dinucleotide phosphate oxidase; NO: Nitric oxide; PI3K: Phosphatidylinositol-3-kinase; TF: Tissue factor; TNF: Tumor necrosis factor; TRAF: Tumor necrosis factor receptor-associated factor; VCAM: Vascular cell adhesion molecule; VEGF: Vascular endothelial cell growth factor; VSMC: Vascular smooth muscle cells.

the highest quintile of resistin plasma levels had a higher incidence of AMI^[109]. The serum resistin concentrations were higher in women, and the associated increase in the risk of AMI based on the resistin level was also higher in women than in men.

In patients with known CAD, some cross-sectional and case-control studies showed higher plasma resistin levels than controls. Subject referring angina which had CAD at the coronary angiography had higher Resistin levels than patients without CAD^[110]. Besides, resistin was also associated with coronary artery calcification at the CT scan^[111]. Pischon *et al*^[112] documented higher resistin levels in women with CAD compared with healthy subjects from the CORA study. This link remained significant even after adjustment for several risk factors except the hs-CRP levels.

In contrast, no relationship between Resistin levels and CAD was found among 1161 subjects in the LURIC study^[113]. Moreover, Resistin was not associated with cardiovascular mortality. Then, high resistin levels could simply mirror the presence of other established cardiovascular risk factors. However in the same study, an enhanced expression of adhesion molecules was found in association with increased resistin levels, highlighting a pathophysiological role in atherogenesis.

In a perspective study^[114] comparing subjects with stable angina and subjects with unstable angina (UA), NSTEMI, and STEMI, higher resistin levels were found within subjects with ACS. Interestingly, an early rise in resistin levels was reported, at 3-6 h after symptoms onset. This increase lasted 6 and 12 h after.

CRP

CRP is an acute phase protein, member of the *pentraxin family*. Since it is a well-known marker of systemic inflammation^[45], CRP was one of the first studied protein from its potential role in both pathogenesis and risk prediction of atherosclerosis. Subsequent studies showed that CRP is other than an innocent bystander of the inflammatory response associated with atherogenesis^[45,115] (Table 2). In particular, together with the Adipocytokines, CRP characterizes the chronic inflammatory status associated with obesity and MetS.

Interestingly, the adipose tissue has been described as producer of CRP^[23,116]. In particular, we found that mature adipocytes are able to produce CRP, under inflammatory stimuli^[23]. This finding was confirmed later in the experience by Anty *et al*^[117], which demonstrated the expression of the CRP gene in adipocytes of obese subjects.

From a clinical point of view, high sensitivity assays are available to detect even low CRP concentration. Then high-sensitivity (hs) CRP has been largely evaluated as a suitable candidate for cardiovascular risk prediction. This idea was first supported by pioneering studies by Ridker *et al*^[118], which demonstrated higher hs-CRP levels in apparently healthy subject who developed CV events during follow-up. In light of these results, Ridker *et al*^[119] evaluated several risk prediction algorithms to improve the cardiovascular risk classification in apparently healthy American women. In particular, a simplified score, including hs-CRP (Reynolds risk score), was validated in this study, and subsequently in a male population^[120].

Then the American Heart Association (AHA) and the CDC Consensus incorporated hs-CRP into the risk prediction strategy of cardiovascular diseases^[121]. Measurement of hs-CRP is considered reasonable in the assessment of absolute risk for CAD in intermediate-risk individuals, with a Framingham risk score of 10% to 20%. This recommendation was confirmed in ACCF/AHA Guidelines in 2010^[122] and in the recently published European Society of Cardiology Guidelines on Prevention^[123].

Moreover, in a metaanalysis^[124] confirmed a role of hs-CRP for a better risk stratification of subjects at intermediate risk for CVD. In particular, for every 400 to 500 people screened for hs-CRP or fibrinogen level, one additional cardiovascular event could be prevented over a period of 10 years.

Results from the Justification for the Use of Statins in Primary Prevention: an International Trial Evaluating Rosuvastatin (JUPITER)^[125] provided robust evidence of the association between inflammation and cardiovascular risk. subjects with LDL cholesterol below 130 mg/dL were treated with rosuvastatin *vs* placebo; patients at higher cardiovascular risk were identified by a hs-CRP level of 2.0 mg/L or higher. The Steering Committee stopped the trial after a median follow-up of 1.9 years due to striking benefit in patients treated with rosuvastatin (44% relative risk reduction of the primary end point and of hard outcomes).

Tehrani *et al*^[126], recently investigate whether inflammatory markers had an impact on the association of high density lipoprotein (HDL) cholesterol with CVD. In 3888 older adults without known CVD, authors evaluated CRP, IL-6, and lipoprotein-associated phospholipase A2 levels. CAD incidence was higher for higher levels of CRP, IL-6, and lower for higher levels of HDL-C. Compared to high HDL-C/low-inflammation categories, incident CAD was increased for those with high HDL-C and high CRP or highest IL-6 tertile. Then the protective relation of high HDL-C for incident CAD appears to be attenuated by greater inflammation.

Hs-CRP has also been studied for its potential role in the prediction of adverse outcome in patients with established CVD. Several studies clearly demonstrated an association between hs-CRP and future acute coronary events in patients with SA^[42].

However, conflicting report exists about the additive benefit of measurement of hs-CRP. While data from Sinning *et al*^[127] suggest that, in patients with established CVD, traditional risk factors are the most powerful predictors with only little information added by inflammatory markers (including CRP), on the other hand several studies^[45,128] showed that hs-CRP independently predicts cardiac events in patients with ACS^[45].

Moreover, patients with higher hs-CRP on admission for ACS had higher rate of impaired myocardial perfusion^[129] and death.

Nakachi *et al*^[130] reported that an hs-CRP elevation at admission and increase independently predicted 30-d events. In contrast, Bogaty *et al*^[131] found that serial mea-

surements of hs-CRP in ACS patients have only a modest predictive ability, which disappeared after adjustment for common clinical variables. However authors did not exclude patients with acute or chronic inflammatory diseases.

Among ACS patients of the FAST-MI, authors found that low fetuin-A and high hs-CRP concentrations were associated with cardiovascular death, even after adjustment for GRACE risk score^[132]. In another study by Schaub *et al*^[133] in 398 consecutive patients presenting with acute chest pain novel biomarkers like myeloperoxidase, MRP-8/14 and hs-CRP, provided incremental value in the risk stratification of these patients.

Apelin

Apelin was first discovered in 1998^[134], as the ligand of the so-called APJ receptor, a G-protein-coupled receptor (GPCR) identified in 1993 from a human genomic library. It is produced as *preproapelin*, then cleaved by an AT-converting enzyme to form several shorter C-terminal active peptides, *i.e.*, apelin-13, -16, -17, -19, -36 and a pyroglutamate form (Pyr1 apelin-13)^[135]. Since the absence of an immediately apparent ligand, APJ was first classified as an *orphan GPCR*. It shares 31% sequence homology with the human angiotensin II (AT II) type 1 receptor, which led to further characterization of the Apelin-APJ system.

Overall, the apelin system has several physiological roles, most notably in the cardiovascular system, hypothalamus and the adipo-insular axis^[136], such as fluid homeostasis, glucose homeostasis, feeding behaviour, regulation of vascular tone, cardiac inotropism and immunity. First studies about apelin-APJ system found both similar and opposite functions to those of the AT system^[137]. The distribution of both receptors and peptides overlaps in the hypothalamus and vasculature^[138]. Moreover, Apelin has been detected in adipose tissue^[139] and it was found that it was both produced and secreted by adipocytes. Apelin has been then considered as a novel adipokine. Also APJ is present in human and mouse adipose tissue, both in isolated adipocytes and in the stromal vascular fraction^[140].

Apelin expression in adipose tissue is regulated by nutritional status. In obese subjects, APJ-apelin expression is increased and this up-regulation could be reversed after diet or surgery-induced weight loss^[141]. Moreover, changes in insulin levels might be involved for both apelin and APJ regulations in adipose tissue, according to the severity of insulin resistance^[140]. A close relationship between apelin and insulin has been demonstrated both *in vivo* and *in vitro*. In cultured adipocytes, insulin treatment increased expression and secretion of apelin. Apelin expression in adipocytes is increased in various mouse models of obesity associated with hyperinsulinemia^[139,142]. Interestingly, in highly insulin-resistant mice, such as db/db ones, APJ expression isn't increased^[143] and in studies conducted in type 2 diabetic subjects, the effect of insulin resulted completely blunted in adipose tissue^[140].

Moreover, Apelin expression in adipose tissue is regulated also by TNF- α , gastro-intestinal inflammation, per-

oxisome proliferator-activated receptor gamma (PPAR γ) coactivator-1 α (PGC1 α), Eicosapentaenoic acid- α polyunsaturated fatty acid from the omega-3 family, which all increase the apelin expression and secretion^[142,144-146]. AT II exerts different effects on the expression of apelin, depending on the receptor involved: type 1 AT receptor mediates an increase of the apelin secretion, while type 2 receptor may reduce its production^[147]. Interestingly, glucocorticoids modulate the production of apelin and its secretion from fat cells, simultaneously increasing AT II and suppressing apelin expression, suggesting a possible pathogenetic mechanism in obesity-related hypertension^[148].

Since the increase in vascular density is essential for adipose tissue expansion, with endothelial cells actively promoting the development of preadipocytes and growth of mature adipocytes, apelin has been proposed to contribute to the development of new vasculature in expanding fat depot^[149]. Several studies have demonstrated that apelin is a potent angiogenic factor^[149], grossly equivalent to vascular endothelial cell growth factor and, like other angiogenic factors, its gene is upregulated under hypoxia condition^[150]. Hypoxia induces expression and secretion of apelin in both human and murine adipocytes, through the hypoxia-inducible transcription factor 1 α .

The first report in humans of plasma apelin concentrations was shown in obese and hyperinsulinemic subjects^[139,141] where plasma apelin levels are increased. In morbidly obese patients with or without diabetes, apelin levels were only higher in the diabetic morbidly obese subjects^[151]. However, reduced plasma apelin levels were described in obese subjects with untreated type 2 diabetes, compared to non-diabetic subjects and anti-diabetic treatment (rosiglitazone and metformin) was found to increase apelin concentration, with the improvement of glycemic profile^[152,153].

Changes in apelin levels after weight loss or bariatric surgery in obese individuals were also investigated. Diet-induced weight loss decreases apelin levels in moderate obese women^[154] but not significantly in patients with the MetS^[155] or in obese children^[156]. Bariatric surgery resulted in a significant decrease in apelin levels only in morbidly obese patients exhibiting impaired fasting glucose or type 2 diabetes before surgery^[151]. Probably, obesity *per se* is not the main determinant of increased plasma apelin concentrations since circulating apelin levels are not necessary significantly correlated to the BMI in all the published studies^[140].

The possible role of Apelin in the atherosclerotic process has been investigated. In studies by Liu *et al.*^[157], Apelin-13, the predominant circulating apelin isoform, significantly promoted intracellular cholesterol efflux and reduces macrophage foam cell formation, indicating a potential antiatherogenic function. Moreover, Kadoglou *et al.*^[158] have demonstrated lower apelin levels in patients with carotid atherosclerosis as compared to healthy controls, and that apelin increment is independently associated with atorvastatin-related carotid plaque stabilization. The same

group reported considerably lower apelin concentrations in CAD patients in comparison with healthy controls^[159]. This finding was confirmed in other studies conducted among subjects with stable angina (SA), and the plasma apelin levels were found to be negatively correlated with the severity of the disease^[160].

A decrease of Apelin levels early after AMI has also been reported, with a progressive elevation over time, however reaching values lower than control subjects at 24 wk^[161]. These observations were confirmed in patients with a first STEMI, where the reduction in apelin levels was independent from left ventricular dysfunction and outcome^[162]. In comparison to asymptomatic CAD patients, plasmatic apelin were lower in ACS patients on admission, with a negative correlation with the severity of CAD^[163].

A myocardial protective effect has been suggested from studies on the possible therapeutic use of apelin in CAD in animal models. Azizi *et al.*^[164] demonstrated in rat models of MI that post-infarct treatment with [Pyr1]-apelin-13 significantly attenuates myocardial damage, *via* the reduction of oxidative injury and enhancement of NO production. In addition, apelin-13 has been found to promote angiogenesis and ameliorate cardiac repair after AMI by a mechanism involving vascular progenitor cells^[165].

However from a pathophysiological point of view, some conflicting data have been reported. For example, Rittig *et al.*^[166] observed that plasma apelin levels are not associated with early stages of atherosclerosis in young subjects prone to atherosclerosis and type 2 diabetes. Interestingly, other studies in animal models suggested a role of apelin-APJ system in vasculature oxidative stress. Furthermore, Apelin is upregulated in human atherosclerotic coronary artery and potentially constricts human coronary artery^[167]. Data by Jin *et al.*^[168] show that genetic defects in apelin/APJ pathway may confer a potential risk for CAD in Chinese hypertensive patients. These evidences underline the complex role of Apelin and its receptor in atherosclerosis.

CONCLUSION

Vascular inflammation represents a fundamental link between obesity, MetS and their detrimental complications. Conflicting evidence about the *in vitro* and *in vivo* effects of Adipocytokines suggests the high complexity of these mediators interplay in the pathogenesis of atherosclerosis and, moreover, in the risk stratification of CAD patients. Even large evidence about the use of hs-CRP for primary and secondary prevention of CVD has been questioning for its real additive value.

However, the involvement of adipocytokines in the pathogenesis of atherothrombosis and dysmetabolism remains clear, although it appears to be way more complex than previously thought. The understanding of these pathways may lead to the development of targeted treatment of obesity-related disorders. In this setting, the

JUPITER trial provided some clue about the association between inflammation and the risk of CVD, even though it was not designed to evaluate the role of the pharmacological modulation of inflammation^[124]. In this context, only two trials are ongoing, the Cardiovascular Inflammation Reduction Trial^[169] and The canakinumab anti-inflammatory thrombosis outcomes study (CANTOS)^[170]. The first is investigating the role of low-dose methotrexate on incident heart attacks, strokes, or death in people with type 2 diabetes or MetS that have had a heart attack or multiple coronary stenoses. CANTOS is studying the effect of Canakinumab, a human monoclonal antibody that neutralizes interleukin-1beta, in secondary prevention^[170].

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WJC 6th Anniversary Special Issues (2): Coronary artery disease

Risks and diagnosis of coronary artery disease in Hodgkin lymphoma survivors

Serhan Kupeli

Serhan Kupeli, Department of Pediatric Oncology and Pediatric Bone Marrow Transplantation Unit, Faculty of Medicine, Çukurova University, 01330 Adana, Turkey

Author contributions: Kupeli S solely contributed to this paper. Correspondence to: Serhan Kupeli, MD, MSc, Associate Professor of Pediatrics, Department of Pediatric Oncology and Pediatric Bone Marrow Transplantation Unit, Faculty of Medicine, Çukurova University, Rektörlüğü, 01330 Adana, Turkey. serhankupeli@cu.edu.tr

Telephone: +90-32-23387444 Fax: +90-32-23387444

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Abstract

Higher mortality rates are reported because of cardiovascular diseases in individuals living in industrialized areas of the World. In cancer patients, cardiotoxic chemotherapeutic agents and/or mediastinal radiotherapy are additional risk factors for the development of coronary artery disease. An improved survival rate for patients with Hodgkin lymphoma was reported in recent decades. Determining and handling the long-term effects of cancer treatment have become more important nowadays, parallel to the good results reached in survival rates. Mediastinal radiotherapy and cardiotoxic chemotherapeutic agents are routinely used to treat Hodgkin lymphoma but are commonly associated with a variety of cardiovascular complications. Drugs used in cancer treatment and radiotherapy may cause deleterious effects on contractile capacity and conduction system of the heart. Approximately ten years after the completion of all therapies, the cardiovascular disease risk peaks in patients who survived from Hodgkin lymphoma. The value of coronary computed tomography angiography as a diagnostic tool in determining coronary artery disease as early as possible is underlined in this review, in patients who are in remission and carry the risk of coronary artery disease probably because of

chemo/radiotherapy used in their treatment. Survivors of Hodgkin lymphoma especially treated with combined chemoradiotherapy at younger ages are candidates for coronary computed tomography angiography.

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Key words: Coronary artery disease; Hodgkin lymphoma; Computed tomography angiography; Cardiotoxicity; Survivors

Core tip: With substantial increase in survival rates from cancer, late adverse effects of cancer therapy have become extremely important. Mediastinal radiotherapy and cardiotoxic chemotherapeutic agents are routinely used to treat Hodgkin lymphoma (HL) but are commonly associated with a variety of cardiovascular complications including coronary artery disease (CAD). For surviving individuals after HL treatment, coronary computed tomography angiography is a non-invasive and useful method in detecting CAD at an early stage. Survivors of HL especially treated with combined chemo-radiotherapy at young ages, who carry the risk of CAD development are candidates for coronary computed tomography angiography.

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INTRODUCTION

Surviving individuals after treatment of malignant diseases have markedly increased in last decades probably because of advanced diagnostic abilities and effective cancer treatment. Long-term unintended effects of aggressive treatments, unfortunately have emerged as a

serious problem at the same time. The adverse effects on heart are among the deadliest effects having high rate of morbidity and mortality. Cardiotoxic chemotherapeutic agents, such as doxorubicin, daunorubicin and epirubicin can decrease the cardiac functioning and contractility of myocardium and the signs of malfunction may even emerge many years after ceseation of cancer treatment^[1-5]. The degree of cardiac dysfunction depends basically on cumulative drug doses of anthracyclines^[6-11]. Mediastinal radiotherapy delivered at the same time with cardiotoxic antineoplastic drugs can also affects the normal functioning of the heart in that population^[12,13]. Screening these individuals for treatment related cardiac toxicity, diagnosing and treating them as early as possible are cornerstones of proper management of cardiovascular disorders. Therefore, screening of cardiac functions of these individuals after ceseation of cancer treatment is particularly important and the principles for the following-up of these patients have been published^[14].

Some researchers have reported higher relative risks of myocardial infarction mortality in patients treated at younger ages than in patients treated at older ages and in men than in women^[15-19]. Other researchers have reported valvular dysfunction, carotid, subclavian and coronary artery disease and even fatality from cardiac infarction at early childhood after radiation therapy for the treatment of Hodgkin lymphoma (HL)^[20-23]. In contrast to numerous papers dealing with cardiac functions in cancer survivors, articles investigating the status of heart and its vasculature in survivors of HL treated in pediatric age group are scarce^[11-13,24].

CORONARY HEART DISEASE

In industrialized Western countries, coronary heart disease is among the leading causes of mortality^[25,26]. Coronary artery disease (CAD) is diagnosed more often in middle-aged males and it is also one of the major causes of mortality in women after menopause^[27]. Advanced biological age, hypertension, increased body-mass index, hyperlipidemia, diabetes mellitus, smoking or use of tobacco products, and presence of CAD among the family members are among the traditional risk factors for CAD^[28]. Researchers are trying to find out genes that creating predisposition to CAD^[29-31]. Beside these, cardiotoxic chemotherapeutic agents and/or mediastinal radiotherapy are additional risk factors for cancer survivors.

RISK FACTORS IN PATIENTS WITH HODGKIN LYMPHOMA

Hodgkin lymphoma

In developed countries, lymphomas are the third most frequent tumors among the pediatric cancers following leukemias and central nervous system tumors. In contrast, in our country and most of the developing parts of the World lymphomas just follow the leukemias in frequency^[32]. With advanced diagnostic and therapeutic fa-

cilities the survival rates in low and high risk patients with HL increased to 95% and 90% respectively^[33,34]. Similarly, improved results after HL treatment were also published in articles from Turkey in recent years^[35,36]. To diagnose earlier and proper treatment of long-term unwanted effects have become one of the main issues in practice of both Pediatric and Medical Oncology parallel to the good results taken in cancer treatment. The frequency of cardiovascular disease peaks generally five to ten years after the completion of HL treatment^[35,36].

Treatment toxicity in HL

In HL combined chemotherapy with low dose involved field radiotherapy (1500-2500 cGy) is the preferred treatment. The mostly used chemotherapeutic regimens in HL are mechlorethamine, vincristine, prednisone and procarbazine (MOPP), cyclophosphamide, vincristine, prednisone and procarbazine (COPP), doxorubicin, bleomycin, vinblastine, dacarbazine (DBVD), OPPA (vincristine, procarbazine, prednisone, adriamycin), and MOPP/DBVD alternating protocols^[33,34]. Among the acute side effects of multiagent chemotherapy protocols nausea and vomiting are the leading ones. Many chemotherapy schemes produce bone marrow suppression and reversible alopecia. Bleomycine-related pulmonary toxicity, vincristine-related neurotoxicity, doxorubicin-related cardiotoxicity are the other side effects of chemotherapy. Radiation pneumonitis, pulmonary fibrosis, spontaneous pneumothorax, abnormalities in growing soft tissues and bones, cardiovascular, and endocrine abnormalities constitute the late effects of treatment. Second malignant neoplasms, especially ALL, are also among the late effects of therapy^[33,34,37-39].

Antineoplastic drugs, especially anthracyclines and mediastinal radiotherapy can cause decrease in cardiac contractility, heart insufficiency, pericardial effusion, constrictive pericarditis, coronary artery disease, myocardial infarction, and arrhythmias^[15-19,40]. Vascular narrowing and cerebrovascular accidents are also among the late complications. Late subclinical cardiovascular side effects are apparent especially in patients 30 to 50 years of age^[37]. The most common chemotherapeutic agents implicated in the development of cardiovascular complications include the anthracyclines, alkylating agents, and vinca alkaloids^[41,42]. Alkylating agents such as cyclophosphamide may exacerbate anthracycline or radiation induced cardiac injury. In adults, the frequency of congestive heart failure increases with the cumulative doxorubicin doses greater than 550 mg/m²^[37]. Mediastinal radiation and other chemotherapeutic drugs are thought to lower the threshold. Since then, all patients treated with anthracycline-containing protocols and mediastinal radiotherapy must be followed up for cardiac injury.

Effect of radiation on vessels

In the treatment of HL, anthracyclines and delivering irradiation to the nodal areas affected are routinely administered. Although not more often, deaths because of myocardial infarction at early ages after HL treatment

were reported^[20-23]. It is impossible to find out exact figures in literature for the frequency of heart diseases in HL survivors. Radiation arteritis may occur as a result of the previous radiation therapy^[43]. Arteries of young children are more susceptible than those in adults. Stenosis and occlusion can be detected angiographically in arteries in the area of radiation. Additionally computed tomography angiography (CTA) can show arterial wall thickening and radiation effects in other soft tissues.

The effects of radiation in tissues received radiation can be classified into a few groups: occurring in epithelial and parenchymal organs, in blood vessels and in stroma^[43]. The vessels having the shortest diameter are the most radiosensitive ones. The reason behind this sensitivity is mostly arising from vulnerable character of endothelium layering the vessels. The changes of radiation in tissues are best studied and documented in animal trials and include irregularity of cytoplasm with the formation of pseudopodia or swelling of cytoplasm often obstructing the lumen, detachment of endothelial cells from the basal lamina, cell pyknosis, rupture of plasma membrane, thrombosis, and rupture of the capillary wall^[44].

Arteritis occurs basically in vessel wall and inflammation progresses to thickening in arterial wall resembling the process of atherosclerosis^[45]. Foam cell plaques in medium and small arteries are suggestive of irradiation. Recent studies confirm that acute vasculitis can be induced by ionizing radiation. Some researchers determined acute vasculitis in small arteries next to coronary arteries or iliac arteries exposed to local radiation therapy. The estimated doses received at the sites of vasculitis varies between 600 and 4000 cGy. Large arteries are less often affected from radiation because of their large lumen and thick wall. Some experimental evidence indicates that arterial perforations may occur due to high dose irradiation^[43].

HL and CAD

Heart diseases are among the frequently seen long-term effects of chemo/radiotherapy used in HL treatment. Mediastinal radiotherapy and cardiotoxic chemotherapeutic agents are commonly associated with a variety of cardiovascular complications including CAD. The mechanism of injury is multifactorial and likely involves endothelial damage of the coronary arteries and secretion of multiple inflammatory and profibrotic cytokines^[46-48]. Heidenreich *et al*^[23] have reported unexpected early deaths from myocardial infarction at young ages after HL^[21-23].

Taken into consideration the relation between the degree of HL treatment and treatment related risks on heart, studies conducted with the aim of giving smaller doses of radiotherapy and lower doses or shortened duration of cardiotoxic agents can limit heart toxicity. Monitoring the patients for classical and generally accepted risk factors for CAD is another important method in lowering the incidence of heart diseases in HL survivors. Rademaker *et al*^[13] reported that coronary CTA and calcium scores are useful methods for the evaluation of irradiation-related CAD in their nine patient series. In a recent study, we investigated CAD by using CTA in 119

HL survivors treated at the pediatric age group^[12]. Hodgkin lymphoma survivors who are in remission at least 2 years after cessation of treatment were investigated. They were questioned about the coronary artery risk factors. Complete blood count, general biochemistry, lipid profile, cardiac troponin-T (cTT) and creatinine kinase myocardial band have been studied. Additionally electrocardiogram (ECG), telecardiography, echocardiography, and coronary CTA were undertaken in all patients. Using a multiplanar reformat, intensity projection, and volume rendering reformat techniques, CTA images were reviewed and mediastinal and cardiac vascular abnormalities were investigated. In 19 (16%) of the patients we determined coronary artery abnormalities. We found statistically significant relation between radiation therapy delivered to the mediastinum and development of an abnormality in coronaries. Probability of developing a coronary abnormality was 6 to 8 times higher in group of patients receiving mediastinal radiotherapy more than 2000 cGy in comparison with the other group receiving radiotherapy less than 2000 cGy by multivariate analysis ($P = 0.009$)^[12]. This study confirms the detrimental late effects of mediastinal radiotherapy on coronary arteries of growing children.

DIAGNOSIS OF CARDIOVASCULAR DISEASES AFTER TREATMENT OF HODGKIN LYMPHOMA

Screening for cardiovascular complications

Screening the long-term survivors of a malignant disease for chemo/radiotherapy related toxicity on heart and managing the abnormalities as early as possible are obviously vital strategies in good management of cardiovascular complications. For this reason, cardiac monitoring of surviving patients after completion of treatment is an obligation.

It is ideal to find out minimally invasive and accurate methods of diagnosis to describe cardiac toxicity similar to other late-effect studies. Currently, most of the centers use echocardiography (ECHO) for periodic follow-up of the heart condition. cTT, an appropriate serological marker to suspect from damage in myocardium was suggested for earlier detection of anthracycline related toxicity after animal studies^[49]. However, no elevation of serum cTT after cessation of adriamycin was reported, although insignificant increases were scored in individuals receiving adriamycin^[50]. Kismet *et al*^[11] have found no correlation between serum cTT values, cumulative dose of adriamycin, and systolic or diastolic functions of the heart and concluded that screening with ECHO is more appropriate than cTT for determining subclinical cardiotoxicity.

Echocardiography is the most commonly used diagnostic facility to follow cardiac functions of cancer survivors^[1]. The traditional approach of screening cardiac toxicity comprises a baseline examination before the start of the cardiotoxic chemotherapy and serial measurements of contractile capacity of the heart (*e.g.*, ejection frac-

tion and fractional shortening) during the course of the treatment. However, the measurement of only ejection fraction as an indicator of left ventricular (LV) function is not reliable to determine subclinical disorders of myocardium^[51,52]. Additionally, conventional Doppler ECHO has some limitations, basically because its dependence on loading conditions, and frequently has negative influence on the interpretation of the findings.

Tissue doppler imaging (TDI) is recently used commonly to evaluate the velocity of myocardial segments with the use of Doppler effect. TDI is superior to traditional Doppler studies in that it can overcome the dependence of loading and detect the abnormalities in LV. This new technique can be employed in evaluation of LV functioning in part or in whole. TDI has some advantages on conventional Doppler ECHO in the evaluation of global or regional diastolic functional capacity of LV^[53]. Alehan *et al*^[24] showed that subtle systolic and diastolic malfunction occurs in long-term survivors of HL by using TDI. Survivors treated with anthracycline based chemotherapy and/or mediastinal radiotherapy may suffer from heart toxicity many years after the cessation of treatment. Malfunction in cardiac systole generally follows the dysfunction in cardiac diastole and prophylactic administration of medications such as angiotensin converting enzyme inhibitors can help preventing the deterioration of heart damage. Obviously, more investigation is necessary to find out accurate strategy for monitoring heart toxicity, but it seems at least today, serial examinations of contractile capacity with TDI in individuals who are in remission after HL treatment can help determining patients under risk of cardiac disorders^[24].

Screening for coronary artery disease with CTA in survivors of Hodgkin lymphoma

CTA employs X-ray to screen blood flow in vasculature in whole body^[54]. X-ray bundles are scattered from a spinning device into the body part which is examined, and they form cross-sectional images that are collected by the computer to give a 3D Picture of the study region. Compared to catheter angiography, the gold standard procedure for evaluation of arteries, involving placement of a catheter and injection of some amounts of contrasted medium into a large vessel, CTA is a minimally invasive procedure. Major and minor complications can be seen in conventional angiography^[55]. Contrasted material is injected into a small vein in CTA, and for most of the patients hospitalization is not necessary. Apart from cost advantage compared to conventional angiography CTA provides information about the vascular wall and soft tissues besides vessel lumen, helps determining the pathologic vessels and additional extra vascular abnormalities in advance^[12,13,56].

In the cardiac CT, predicted radiation exposure is 2-2.5 Rem and this is higher than 1.5-2 Rem that is exposed in diagnostic pediatric cardiac catheterization^[54]. With contemporary modern detectors, the exposure can be decreased by using the ECG dose modulation technique by using higher X-ray doses to evaluate coronary arteries in

diastolic phases and lowered doses in systolic phases^[21]. In normal coronaries, it is unusual to find calcification in an arterial wall. CTA is also sensitive in detecting calcium in arterial wall^[13]. The increase in calcium scores can be halted with the use of hypolipidemic drugs in patients with high calcium scores in their coronaries^[57]. A conventional angiography, however, cannot be indicated solely based on coronary calcium scoring due to its low specificity^[55]. In the presence of massive coronary calcification, a CTA cannot show the thickening in the vessels because of signal changes^[54].

Although the CTA has found a place of application in many fields and clinical situations^[58-61] it currently has some limitations. Blocked blood vessels make difficult the interpretation of the images^[55]. The CTA is not yet reliable for visualization of small, vessels in rapidly moving organs. CTA images can be blurred because of movements during the examination or because of the heart that is not beating properly. High-density objects such as metal clips, stents, and calcified plaques prevent the proper visualization of the neighboring tissues by the attenuation they created^[55]. The dose of radiation exposed during the examination is also a limiting parameter. With a 64-detector computerized tomography, the dose of radiation given to the patients is approximately 6.5 to 15 mSv and this is much more than that used in conventional angiography^[54]. The examination brings some risks such as allergic reaction to the contrast material and it must not be performed in renal disease, severe diabetes, pregnant or breastfeeding women.

The above mentioned study is the unique study in which CTA was used for determination of abnormalities in coronary arteries in HL survivors treated in childhood^[12]. The capability of CTA in early detection of CAD was shown for the first time in this patient population. Based on our findings we concluded that individuals at the age of 17-28 years, treated in childhood for HL and carry the risk of CAD and specifically treated with radiation therapy into the mediastinum, are candidates for coronary CTA.

CONCLUSION

Serial follow-up including screening for valvular disease with TDI and coronary artery disease with CTA and coronary artery calcium scoring, must be applied to the survivors of HL who have been treated with anthracycline including regimens and/or mediastinal radiotherapy like a great majority of the patients with HL.

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Non-invasive evaluation of arrhythmic risk in dilated cardiomyopathy: From imaging to electrocardiographic measures

Massimo Iacoviello, Francesco Monitillo

Massimo Iacoviello, Francesco Monitillo, Cardiology Unit and Cardiothoracic Department, Policlinico Consorziale University Hospital, 70124 Bari, Italy

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Correspondence to: Massimo Iacoviello, MD, PhD, Cardiology Unit and Cardiothoracic Department, Policlinico Consorziale University Hospital, Piazza Giulio Cesare 11, 70124 Bari, Italy. massimo.iacoviello@policlinico.ba.it

Telephone: +39-08-05478622 Fax: +39-08-05478796

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Core tip: Arrhythmic risk stratification and decision making towards implantation of a cardioverter defibrillator in dilated cardiomyopathy patients are still open challenges. This review critically revises the possible clinical usefulness of available non-invasive diagnostic tools employed to stratify arrhythmic risk prognosis in dilated cardiomyopathy patients.

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Abstract

Malignant ventricular arrhythmias are a major adverse event and worsen the prognosis of patients affected by ischemic and non-ischemic dilated cardiomyopathy. The main parameter currently used to stratify arrhythmic risk and guide decision making towards the implantation of a cardioverter defibrillator is the evaluation of the left ventricular ejection fraction. However, this strategy is characterized by several limitations and consequently additional parameters have been suggested in order to improve arrhythmic risk stratification. The aim of this review is to critically revise the prognostic significance of non-invasive diagnostic tools in order to better stratify the arrhythmic risk prognosis of dilated cardiomyopathy patients.

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Key words: Dilated cardiomyopathy; Major ventricular arrhythmias; Prognosis; Ventricular repolarization; Left ventricular systolic function

INTRODUCTION

The main adverse events affecting the prognosis for both ischemic (IDCM) and non-ischemic (NIDCM) dilated cardiomyopathy patients are the occurrence of malignant ventricular arrhythmias and sudden death and the progression towards heart failure^[1]. In order to reduce the incidence of sudden death due to ventricular arrhythmias, the best therapeutic strategy to date is cardioverter defibrillator implantation (ICD)^[1-3]. Both for NIDCM and IDCM, the decision to implant an ICD is mainly guided by the evaluation of left ventricular systolic function, *i.e.*, by the calculation of left ventricular ejection fraction (LVEF)^[4]. However, its use in defining eligible patients has a number of limitations.

In particular, there are a large number of patients who do not benefit from ICD^[5]. In fact, the majority of patients with low LVEF who were enrolled in the main trials evaluating the effect of ICD did not suffer from malignant ventricular arrhythmias. For example, only 26% of the MADIT II patients had malignant ventricu-

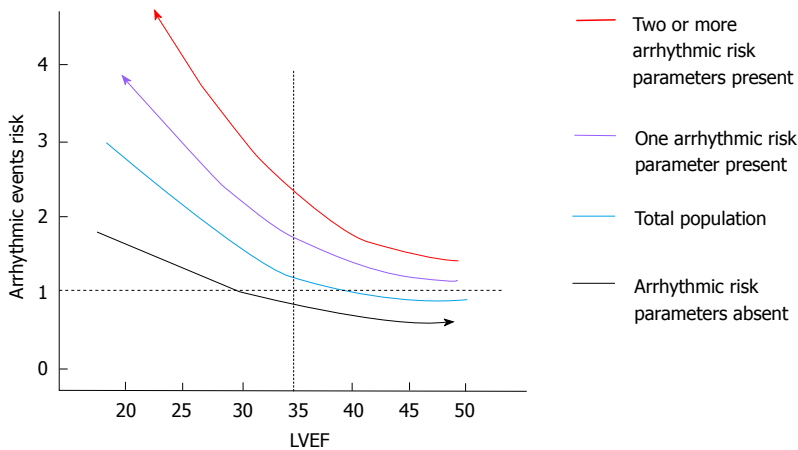


Figure 1 The effect of a better arrhythmic risk stratification are shown. The presence of one or more arrhythmic risk factor allows detection of a population at higher risk of arrhythmic events across all the values of left ventricular ejection fraction. On the other hand, the absence of arrhythmic risk factors is associated with the detection of the group of patients at lower risk of events. LVEF: Left ventricular ejection fraction.

lar arrhythmias during a 24 mo follow-up^[2]. Only 31% of the 829 patients enrolled in the ICD group of the SCD-HeFT trial received shocks from their device for any cause and only 177 (21%) received shocks to arrest rapid ventricular tachycardia or ventricular fibrillation. During a five year follow-up, the annual average rate of ICD shocks was 7.5%; however, the annual average rate for appropriate ICD shocks (*i.e.*, shocks for rapid, sustained ventricular tachycardia or fibrillation) was 5.1%. Moreover, in the SCD-HeFT trial, 32 (4%) patients had their ICD removed during follow-up and ICD complications, defined as clinical events requiring surgical correction, hospitalization, or new and otherwise unanticipated drug therapy, occurred in 5% of patients at the time of implantation and in 9% at a later stage in the trial^[3].

It is clear from these data that the need to better assess arrhythmic risk is still a challenge^[5]. Better characterization of patients using additional parameters should be able to detect those with a higher or lower risk of arrhythmic events, thus avoiding ICD implantation in patients with low LVEF at low risk and facilitating the implantation of patients with good LVEF at higher risk (Figure 1).

The aim of this review is to critically revise the possible clinical usefulness of the available non-invasive parameters related to the pathophysiology of ventricular arrhythmias (Figure 2) which have been proposed in order to better stratify the arrhythmic risk of dilated cardiomyopathy patients.

THE IMAGING TO DETECT ARRHYTHMIC SUBSTRATES

The assessment of left ventricular systolic function

As previously stated, the use of LVEF to guide decisions on whether to implant ICD leads to only a small percentage that will suffer from ventricular arrhythmias in a selection of a large population. However, the limitation of this approach is also related to several technical and biological aspects.

Firstly, in repeated evaluations, the LVEF calculation is characterized by a wide variability, particularly when an echocardiographic approach is considered. This is even

more pronounced when different readers perform the calculation^[6]. An improvement in the accuracy of LVEF calculation by echocardiography could be obtained using contrast echocardiography^[7] or the 3-dimensional (3D) approach^[8], but the gold standard for a more accurate and reproducible 3-D quantification of left ventricular (LV) volumes is cardiac magnetic resonance (CMR)^[7,9].

Apart from the technical limitations in LVEF assessment, variability of the measure may also be influenced by biological factors. In particular, LVEF can vary in the different loading conditions due to changes in intravascular volumes and/or adrenergic drive^[5,10]. Moreover, LVEF can change over time in response to conventional medical therapy^[11].

In this setting, the new echocardiographic measures to evaluate left ventricular systolic function, which are less loading dependent, could be a new, useful tool to improve arrhythmic risk stratification by echocardiography^[10]. Among these, two-dimensional (2-D) speckle tracking analysis^[12] seems to be a particularly promising technique as it has been validated by sonomicrometry and tagged magnetic resonance imaging^[13] and can quantify global and regional cardiac function more accurately and objectively by detecting mild ventricular function abnormalities in both left and right ventricular cardiomyopathies^[14-15].

2-D speckle-tracking analysis is based on the detection and the motion tracking of natural acoustic myocardial reflections and interference patterns within an ultrasonic window. The tracking system analyses of echocardiographic grayscale B-mode images permits measurement of the entity of myocardial deformation (strain). Strain parameters can be individualized for each of the myocardial segments or can be expressed as global strain when all the segmental values are averaged. The global longitudinal strain (GLS) is the mean values of myocardial segmental deformation, evaluated using standard apical views. From a technical point of view, the use of 2-D strain measures offers some advantages over routine echocardiographic assessment of LVEF using Simpson's rule. In particular, strain analysis is not based on any geometrical assumption and should depend less on loading conditions. Moreover, in regional contractility

Triggering and/or favouring factors

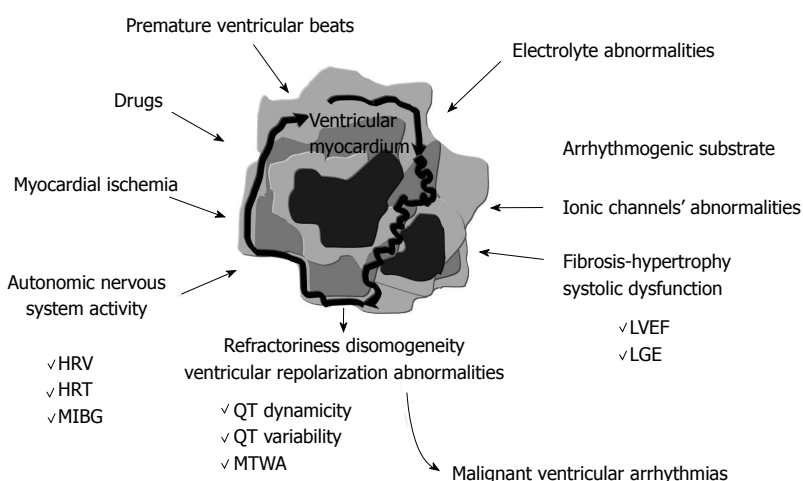


Figure 2 The main parameters proposed in order to better characterize arrhythmic risk are shown. These parameters can reflect arrhythmic substrate by functional (left ventricular systolic function) or anatomical (myocardial fibrosis) information. The parameters assessing sympathetic nervous system activity are also reported, as well as those reflecting the dispersion of ventricular refractoriness, *i.e.*, those based on the analysis of ventricular repolarization. HRV: Heart rate variability; HRT: Heart rate turbulence; LGE: Late gadolinium enhancement; LVEF: Left ventricular ejection fraction; MIBG: Iodine-123 metaiodobenzylguanidine; MTWA: Microvolt T-wave alternans.

dysfunction, strain measures better correlate with LVEF as assessed by magnetic resonance^[16]. Finally, GLS is easy to compute and less dependent on specific training to ensure reproducibility^[17].

In order to evaluate the role of this novel technique in stratifying arrhythmic risk prognosis, we recently studied a group of heart failure (HF) outpatients affected by IDCM and NIDCM who had never previously experienced sustained ventricular arrhythmias^[18]. During a mean follow-up of 26 ± 13 mo, 31 of 230 patients experienced entricular ventricular tachycardia (VT)/fibrillation (VF) or sudden death. At multivariate analysis, after correction for the univariate predictors, *i.e.*, NYHA class, NT-proBNP and non-sustained ventricular tachycardia (NSVT), GLS remained significantly associated with ventricular arrhythmic events. The best GLS cut-off value detected by ROC curves for the 1 year occurrence of events was -10.0% , with a 73% sensitivity and a 61% specificity in detecting patients prone to experiencing major ventricular arrhythmias. Interestingly, the annual incidence rates of arrhythmic events were significantly greater in the 24 patients with a LVEF $> 35\%$ and a GLS above -10% than in the 114 patients with GLS below -10% , whereas no additive value was observed among patients with a LVEF $\leq 35\%$.

Assessment of myocardial fibrosis

In arrhythmic risk stratification, the usefulness of CMR is related not only to the possibility of more accurately estimating LVEF^[19-22], but also to its ability to detect the presence of myocardial replacement fibrosis^[23]. CMR assessment of fibrosis is made possible by using late gadolinium enhancement. Gadolinium is a contrast agent that has been shown to be extremely safe. It is an extracellular agent, accumulating in areas of interstitial expansion due to myocardial fibrosis, edema or infiltration. After gadolinium administration, it is possible to assess three phases: the first provides immediate images at rest or during stress, followed by early enhancement after 5 min and late enhancement 5 to 20 min after administration^[22]. Late gadolinium enhancement (LGE) imaging allows the detection of contrast accumulation in areas of

infarction or fibrosis due to slower contrast kinetics and greater volume or distribution in extracellular matrix. The extent and pattern of LGE enhancement varies according to the underlying pathological process. Fibrosis extent can be quantified as a percentage of total LV mass using dedicated software^[22-23]. Moreover, the relative safety of gadolinium agents and tissue characterization sequences allows repeated imaging, follow-up, family screening and serial risk stratification^[24].

The presence of fibrosis, as assessed by LGE, is associated with a greater probability of inducible ventricular tachycardia^[25]. Moreover, there is considerable evidence that it is also associated with a worse prognosis and an increased arrhythmic risk. Table 1 summarizes the main studies with this evidence^[26-33].

Assomull *et al.*^[26] first evaluated the prognostic impact of midwall fibrosis in patients diagnosed with NIDCM, prospectively followed up for 658 ± 355 d. Midwall fibrosis was present in 35% of patients and was associated with a higher rate of all-cause death and hospitalization for a cardiovascular event. Multivariate analysis showed that it was the only significant predictor of death or hospitalization. Midwall fibrosis also predicted sudden cardiac death (SCD) or VT and remained predictive of SCD/VT after correction for baseline LVEF.

Iles *et al.*^[28] prospectively evaluated 103 patients meeting criteria for ICD implantation for primary prevention of SCD who were affected by both IDCM and NIDCM. Regional fibrosis was identified with LGE in 71% of patients, in all patients with a diagnosis of IDCM and in 51% of those affected by NIDCM. Interestingly, among NIDCM patients, LGE was associated with arrhythmic events during follow-up in 29%, whereas no NIDCM patients without LGE experienced arrhythmic events.

Finally, the relevant role played by LGE in arrhythmic risk stratification has been supported by a study evaluating a large sample of NIDCM patients^[33]. In this series, 30% of patients had fibrosis and were characterized by a lower LVEF and a more severe functional limitation. The presence of fibrosis was independently associated with an increased arrhythmic risk as well as an increased prob-

Table 1 The main studies evaluating the association between myocardial fibrosis assessed by cardiac magnetic resonance and the risk of arrhythmic and non-arrhythmic events

Ref.	Clinical setting	Number of patients	CMR parameters	End-points (mean follow-up)	Results
Assomoul <i>et al</i> ^[26] , 2006	NIDCM	101	Midwall fibrosis (LGE)	All-cause death and hospitalization (follow-up 658 ± 355 d)	Independent association with death and hospitalization
Wu <i>et al</i> ^[27] , 2008	NIDCM and LVEF ≤ 35%	65	Presence and extent of LGE	Composite end-point (hospitalization for heart failure, appropriate ICD firing, cardiac death) (Follow-up median 24 mo)	Presence of LGE was associated with a greater risk of primary outcome
Iles <i>et al</i> ^[28] , 2011	IDCM/NIDCM before ICD implantation	103	Regional fibrosis with LGE	Arrhythmic events and appropriate ICD therapy (follow-up 573 d)	LGE was associated with arrhythmic events and appropriate ICD therapy during follow-up
Lehrke <i>et al</i> ^[29] , 2011	NIDCM	184	Presence of LGE	Composite end-point (hospitalization for decompensated heart failure, cardiac death, cardioverter defibrillator discharge) (follow-up 31 mo)	Presence of LGE was associated with composite endpoint
Gao <i>et al</i> ^[30] , 2012	IDCM/NIDCM	124	Presence and quantification of LGE	Primary composite outcome: occurrence of appropriate ICD therapy, SCA, SCD (follow-up 632 ± 262 d)	Myocardial scar quantification by LGE-CMR predicts arrhythmic events in patients being evaluated for ICD eligibility
Neilan <i>et al</i> ^[31] , 2013	NIDCM	162	Presence and quantification of LGE	Major adverse cardiac events (cardiovascular death and appropriate ICD therapy) (follow-up: 29 ± 18 mo)	Presence of LGE was a strong predictor of major cardiac events
Li <i>et al</i> ^[32] , 2013	NIDCM	293	Presence and extent of LGE	All-cause mortality (follow-up: 3.2 yr)	Presence of LGE is an independent predictor of increased all-cause mortality Diffuse LGE is associated with higher mortality
Gulati <i>et al</i> ^[33] , 2013	NIDCM	472	Presence and extent of midwall fibrosis	Primary end-point: all cause mortality Secondary end-point: cardiovascular mortality or cardiac transplantation Arrhythmic and HF secondary end-points (follow-up 5.3 yr)	Midwall fibrosis assessed with LGE-CMR provided independent prognostic information and improved risk stratification beyond LVEF for all-cause mortality and SCD

CMR: Cardiac magnetic resonance; IDCM: Ischemic dilated cardiomyopathy; LGE: Late gadolinium enhancement; NIDCM: Non ischemic dilated cardiomyopathy; SCA Survived cardiac arrest; SCD: Sudden cardiac death; ICD: Implantable cardioverter defibrillator.

ability of death. Moreover, whether fibrosis was present or not, it was possible to detect the group of patients at higher and lower risk across the LVEF spectrum. For example, patients with a LVEF of 35% and fibrosis had a 19.9% estimated risk of death *vs* 9.4% of patients with the same LVEF but without fibrosis.

Although there is considerable evidence to suggest the relevance of LGE in arrhythmic risk stratification, particularly in NIDCM, this technique has not been recommended yet by current guidelines for the selection of patients who will benefit from ICD implantation.

ELECTROCARDIOGRAPHIC MEASURES OF ARRHYTHMIC RISK

Fragmented QRS

Prolonged QRS duration prevalence in patients with congestive heart failure varies between 20% and 50%^[34]. Left bundle branch block and, in general, QRS prolongation (> 120 ms) in heart failure patients independently predict increased overall mortality and SCD^[35-36].

However, fragmented QRS complexes (f-QRS) on a routine 12-lead electrocardiogram have also been pro-

posed as a marker of depolarization abnormality^[37].

Various studies have suggested that the region of a myocardial scar is associated with alteration in QRS morphology, leading to a terminal conduction delay or a fragmentation of QRS complexes on the 12-lead ECG^[38-39].

Fragmented QRS includes various RSR' patterns with different morphologies of the QRS interval (QRS duration < 120 ms), with or without the Q wave. It is defined by the presence of an additional R wave (R') or notching in the nadir of the S wave, or the presence of > 1 R' wave (fragmentation) in 2 contiguous leads, corresponding to a major coronary artery territory^[40].

Brenyo *et al*^[41] observed that fragmented QRS (f-QRS), particularly when present in inferior leads, is predictive of SCD, SCD or appropriate ICD shock and all-cause mortality in patients with IDCM.

Sha *et al*^[42] evaluated a population of 128 patients with NIDCM and left ventricular dysfunction (ejection fraction, EF ≤ 40%). They observed that in the group with f-QRS, all-cause mortality and ventricular tachyarrhythmias were significantly more frequent than those observed in the non-fQRS group.

Finally, Das *et al*^[43] tried to assess the prognostic

Table 2 Main studies evaluating the role of dynamic ventricular repolarization measures in predicting arrhythmic and non arrhythmic events

Ref.	Clinical setting	Number of patients	Parameter evaluated	Cut-off suggested	End-points (mean follow-up)	Results
Chevalier <i>et al</i> ^[46] , 2003	Acute myocardial infarction	265	QT dynamicity and HRV (24-h Holter) LVEF Late potential	QTe slope: 0.18	Sudden death and total mortality (follow-up 81 ± 27 mo)	Increased diurnal QTe dynamicity independently associated with sudden death
Haigney <i>et al</i> ^[47] , 2004	Postinfarction patients (low LVEF)	871	QT variability (QTVN) QTVI (QTVN adjusted for heart rate variance)		Arrhythmic events (VT or VF) (follow-up 2 yr)	Increased QT variability associated with an increased risk for VT/VF
Jensen <i>et al</i> ^[48] , 2005	Postinfarction patients	481	QT/RR slope and intercept QT/RR VR LVEF VPB and VT		All-cause mortality (follow-up 3 yr)	VR, LVEF, VPB and age made up the optimal Cox model for risk stratification. VR was a promising risk factor for identifying sudden arrhythmic death
Iacoviello <i>et al</i> ^[49] , 2007	NIDCM (no history of SVT/VF)	179	QTe slope (24 h Holter) LVEF NSVT QRS duration QTe and QTd at ECG	QTe-slope: 0.19	Major arrhythmic events, (VT or VF or SCD) (follow-up 39 ± 22 mo)	Increased QTe slope is associated with occurrence of major arrhythmic events. The presence of NSVT and/or QTe slope > 0.19 showed 90% sensitivity and 60% specificity in identifying patients with arrhythmic events
Cygankiewicz <i>et al</i> ^[50] , 2009	CHF patients. IDCM/NIDCM LVEF ≥ 35%	294	QTe slope SDNN TS LVEF	QTe slope: 0.21	Primary endpoint: total mortality Secondary endpoint: sudden death (follow-up 44-mo)	Combination of SDNN, TS, and QTe slope is a predictor of increased risk of mortality and sudden death

BRS: Baroreflex sensitivity; CHF chronic heart failure; EPS Electrophysiological study; ICD Implantable cardioverter defibrillator; IDCM: Ischemic dilated cardiomyopathy; HR Heart rate; HRV: Heart rate variability; LVEF: Left ventricular ejection fraction; MCE: Major cardiac events; NIDCM: Non ischemic dilated cardiomyopathy; NSVT: Non sustained ventricular tachycardia; OHR: Onset heart rate; QTc: QT interval corrected for heart rate; QTe: QT interval calculated at the end of T-wave; SCD: Sudden cardiac death; SDNN: Standard deviation of RR intervals; SR: Sinus rhythm; TS: Turbulence slope; PVB: Premature ventricular beats; VT: Ventricular tachycardia; VR: Variability ratio.

significance of fQRS for an arrhythmic event in 368 patients with IDCM and NIDCM who underwent ICD implantation for primary or secondary prevention of SCD. The authors concluded that fQRS on a 12-lead ECG is a predictor of arrhythmic events but is not associated with a greater probability of death.

Analysis of ventricular repolarization

The analysis of ventricular repolarization is an intriguing way to implement risk stratification of major arrhythmic events. However, in a large study evaluating NIDCM, the electrocardiographic measure of QT intervals and their dispersion at ECG failed to demonstrate any role in predicting arrhythmic events^[44].

Compared to the “static” evaluation of QT interval and dispersion at ECG, the possibility of evaluating QT dynamicity and/or variability during a short-term or 24 h period offer a more complete assessment of ventricular depolarization, the expression of the complex interaction between arrhythmic substrate, heart rate and autonomic nervous system activity^[45]. Table 2 summarizes the main studies evaluating the prognostic role of QT-dynamicity or variability measures^[46-50].

Recently, we studied a series of patients affected by

NIDCM to evaluate the role of QT dynamicity in predicting major arrhythmic events as assessed by 24-h ECG recordings^[49]. The QT dynamicity index proposed was QT-slope, *i.e.*, the slope of the regression line between QT end and RR during a 24 h period. At univariate analysis, QTe-slope was significantly associated with major arrhythmic events as well as LVEF, NSVT and standard deviation of RR intervals (SDNN). At multivariate analysis, only the QTe-slope, LVEF and NSVT were significant predictors of events, regardless of SDNN, a QRS duration >120 ms or beta-blocker therapy.

The analysis of QT dynamicity has also been found to be associated with an increased arrhythmic risk in patients with IDCM. Chevalier *et al*^[46] demonstrated that QTe slope compared with LVEF, HRV and late potentials was the strongest independent predictor of sudden death in patients with myocardial infarction. In 871 postinfarction patients with severe left ventricular dysfunction enrolled in the MADIT study, Haigney *et al*^[47] demonstrated an increased incidence of malignant ventricular arrhythmias in those with increased QT variability. In this study, QT variability was assessed using a semiautomated algorithm that measured beat-to-beat QT duration. Similarly, in a population of postinfarction patients, Jensen *et al*^[48]

demonstrated the prognostic usefulness of a novel QT dynamics parameter: the QT/RR variability ratio (VR), defined as the ratio between the standard deviation of all QT intervals and the standard deviation of all RR intervals. It was evaluated in 481 patients and found to be associated with the occurrence of sudden arrhythmic death.

Finally, the potential usefulness of QT-e slope has also been demonstrated in a large population of 294 patients affected by CHF due to both IDCM and NIDCM and relatively preserved LVEF $> 35\%$ ^[50].

Microvolt T-wave alternans

Microvolt T-wave alternans (MTWA) analysis involves the detection of changes in T-wave morphology occurring on an every-other-beat basis. A wide electrical alternans of T-wave was an ECG abnormality, first described 50 years ago as being associated with cardiac mortality^[51-52]. Discordant alternans is responsible for dispersion of repolarization of sufficient magnitude to cause unidirectional block and re-entry. A critical dispersion of repolarization is an important condition for development of re-entrant arrhythmias^[53].

Since MTWA is heart rate dependent, it is generally assessed by increasing heart rate with atrial pacing or by exercise stress. The analysis is based on the alignment of ECG cycles to the QRS complex and on the measurement of T-wave amplitude. The beat-to-beat fluctuations of T-wave are then analyzed using fast Fourier transformation and MTWA is represented by the pronounced peak visible in the spectrum at 0.5 cycles/beat. A significant MTWA is present if the alternans voltage is over a threshold (generally 1.9 microV) and if the alternans ratio $K \geq 3$. Generally, an alternans which is longer than 1 min occurring at a heart rate ≤ 110 beats/min is considered positive^[54].

In 1994, Rosenbaum *et al.*^[55] was the first to demonstrate the efficacy of MTWA in stratifying patients for the risk of ventricular tachyarrhythmic events. However, the studies published to date are not concordant, as summarized in Table 3^[56-64].

The meta-analysis carried out by Hohnloser *et al.*^[65] suggested that MTWA assessed by spectral analysis provides an accurate means of predicting major ventricular arrhythmias. Moreover, the event rate was very low among patients with a negative MTWA test. These results were concordant with the meta-analysis by Calò *et al.*^[66] who analyzed fifteen studies involving 5681 patients. A positive MTWA determined an approximately 2.5-fold higher risk of cardiac death and life-threatening arrhythmia and showed a very high NPV in both ischemic and non-ischemic patients. An abnormal MTWA test was associated with a 5-fold increased risk for cardiac mortality in the low-indeterminate group and about a 6-fold increased risk in the beta-blocker group. The potential usefulness of MTWA has also been confirmed by Merchant *et al.*^[67] who analyzed the data of five studies with 2883 patients without ICDs. Among patients with an LVEF of $\leq 35\%$, a negative MTWA test result was associated with a low risk

for SCD. Conversely, in patients with a LVEF of $> 35\%$, a positive MTWA test result identified those at a significantly heightened SCD risk. Finally, the Alternans Before Cardioverter Defibrillator (ABCD) trial^[64] was the first to use electrophysiological study (EPS) or MTWA to guide prophylactic ICD implantation in patients with a LVEF $\leq 40\%$, coronary artery disease and NSVT. The authors demonstrated that risk stratification strategies using the non-invasive MTWA are comparable to invasive strategy.

These results seem to encourage the use of MTWA testing in patients who do not have ICDs in order to identify those at higher risk of ventricular arrhythmic events. However, the meta-analysis of Gupta *et al.*^[68] concluded that spectrally derived MTWA testing does not sufficiently modify the risk of VTE to change clinical decisions. Moreover, the MTWA technique is characterized by limitation in its feasibility. In an unselected population of 1003 patients with HF, Kraaier *et al.*^[69] showed that only half were eligible for MTWA testing and the most common result was an indeterminate test. They concluded that MTWA treadmill testing is not widely applicable in typical HF patients and is unlikely to refine risk stratification for sudden death on a population level.

ASSESSMENT OF AUTONOMIC NERVOUS SYSTEM ACTIVITY

In the genesis of malignant arrhythmias, apart from the presence of a vulnerable substrate, an altered sympathetic nervous activity and the presence of trigger factors, such as ventricular beats, play a fundamental role. The importance of autonomic dysfunction in increasing the risk of death in patients with heart disease may be applicable to all patients with cardiac disease regardless of etiology^[70,71]. The pro-arrhythmic effects of the sympathetic nervous system in the normal and ischemic heart are mainly related to the indirect and direct effects of beta-adrenergic receptor activity, but also to the direct effects of alpha-1 adrenergic receptors activity^[72].

The direct effects on myocardiocytes are mediated by the activation of cyclic nucleotide and protein kinase regulatory cascade, which can alter spatial heterogeneity of calcium transients and consequently increase the dispersion of repolarization^[73]. The major indirect effect of beta-receptors activity is the impairment of oxygen supply caused by increased metabolic activity, coronary vasoconstriction, especially in vessels with damaged endothelium, and changes in preload and afterload. On the other hand, the increase in parasympathetic activity is able to modulate ventricular arrhythmias by means of one of the following three effects: a reduction in sinus heart rate, a direct influence on myocardial electrophysiology and a reduction in myocardial oxygen demand due to the negative inotropic action. However, vagal and sympathetic effects cannot be considered in isolation. Sympathovagal interactions are critical in order to understand the electrophysiological function of the heart. Processes disturbing sympathovagal balance have the potential to facilitate cardiovascular instability, leading to cardiac arrhythmias or

Table 3 Main studies evaluating the role of microvolt T-wave alternans in predicting arrhythmic and non arrhythmic events

Ref.	Clinical setting	Number of patients	Parameter evaluated	End-points (mean follow-up)	Results
Adachi <i>et al</i> ^[56] , 1999	NIDCM	57	TWA, LVEF, NYHA, Signal average ECG, QT dispersion	Ventricular tachycardia	MTWA associated with VT
Klingenheben <i>et al</i> ^[57] , 2000	CHF (no history SVT/VF)	107	TWA	Arrhythmic events (follow-up 18 mo)	MTWA is an independent predictor of arrhythmic events
Kitamura <i>et al</i> ^[58] , 2002	NIDCM	146	Onset heart rate for TWA	SCD, documented sustained ventricular tachycardia/ventricular fibrillation (follow-up 21 ± 14 mo)	TWA and LVEF were independent predictors of arrhythmic events
Hohnloser <i>et al</i> ^[59] , 2003	NIDCM (LVEF 29 ± 11%)	137	MTWA, FEVS, mean RR interval, HRV, BRS.	SCD, SCA, SVT or VF (follow-up 14 ± 6 mo)	MTWA is an independent predictor of ventricular tachyarrhythmic events
Bloomfield <i>et al</i> ^[60] , 2004	IDCM (LVEF ≤ 30%)	177	MTWA, QRS measurement	All-cause mortality. (follow-up 20 ± 6 mo)	Compared to QRS duration, an abnormal MTWA is a stronger predictor of death
Salerno-Uriate <i>et al</i> ^[61] , 2007	NIDCM (NYHA II-III LVEF ≤ 40%)	446	TWA, VO2 peak	Combined primary endpoint of cardiac death and life-threatening ventricular arrhythmias Secondary endpoint: total mortality, combination of arrhythmic death and life-threatening arrhythmias. (follow-up 18 to 24 mo)	Abnormal TWA associated with a 4-fold higher risk of cardiac death and life-threatening arrhythmias
Baravelli <i>et al</i> ^[62] , 2007	NIDCM (NYHA II-III LVEF 29 ± 6.4%)	70	MTWA, VO2 peak	Combined primary endpoint of MCE: total cardiac death or VT/VF (including appropriate ICD shock) Secondary endpoint: MAE: SCD or SVT/VF (follow-up 19.2 ± 10.7 mo)	MTWA and peak VO2, but not the two single tests, were significant prognostic markers of both MCE and MA
Gold <i>et al</i> ^[63] , 2008	CHF (IDCM/NIDCM, 71% NYHA II, LVEF 24 ± 7%)	490	TWA	Composite primary end point: SCD, SVT / VF, or appropriate ICD discharge (follow-up 30 mo)	MTWA not predictive of MAE or mortality
Costantini <i>et al</i> ^[64] , 2009	IDCM LVEF ≤ 40%	566	TWA, EPS	Primary endpoint: appropriate ICD discharge or SCD at 1 yr follow-up (follow-up 1.6 ± 0.6 yr)	Strategies employing MTWA, EPS, or both might identify the subset of patients least likely to benefit from ICD implantation

BRS: Baroreflex sensitivity; CHF: Chronic heart failure; EPS: Electrophysiological study; HR: Heart rate; HRV: Heart rate variability; ICD: Implantable cardioverter defibrillator; LVEF: Left ventricular ejection fraction; MCE: Major cardiac events; MTWA: Microvolt T-Wave alternans; NYHA: New York Heart Association; NSVT: Non sustained ventricular tachycardia; OHR: Onset heart rate; SR: Sinus rhythm; SCA: Sudden cardiac arrest; SCD: Sudden cardiac death; SDNN: Standard deviation of RR intervals; VF: Ventricular fibrillation; VT: Ventricular tachycardia.

even sudden death.

It is clear that every marker of autonomic activity may be used as a clinical prognostic factor. The evaluation of sympathetic nervous system activity can be based on electrocardiographic measures reflecting autonomic control of heart rate, such as the beat-to-beat heart variability (HRV), heart rate turbulence (HRT) and the reflex chronotropic response to a blood pressure change; *i.e.*, baroreflex activity (BRS). Moreover, nuclear imaging techniques can estimate cardiac denervation.

Measures of autonomic control of heart rate

The prognostic role of measures evaluating autonomic control of heart rate has been widely investigated.

HRV is a term which includes a large number of different indices evaluating the beat-to-beat variability by using either time domain or frequency domain analysis^[74]. Time domain analysis is based on the detection of

each QRS complex and on measurement of all intervals between adjacent QRS complexes, resulting from sinus rhythm, as NN intervals or as instantaneous heart rate. Among the statistical time domain indices, SDNN is the simplest and is the standard deviation of NN intervals generally assessed in 24 h Holter recordings. The prognostic significance of SDNN has been evaluated both in patients with ischemic and non-ischemic diseases, as well as in heart failure patients, but the results are controversial.

Brower *et al*^[75] assessed the prognostic value of HRV measures in patients with mild or moderate chronic heart failure (NYHA class II-III). Ninety-five patients were followed-up for 4 years. None of the conventional time and frequency domains were related to survival. Szabò *et al*^[76] followed-up a group of 159 patients with idiopathic or ischemic dilated cardiomyopathy, selected on the basis of a left ventricular ejection fraction of < 40%. During follow-up, cardiac mortality was subdivided into sudden

cardiac death and death due to progressive pump failure. SDNN was found to have an independent predictive value for all cause mortality, while not being related to the type of the death. Fauchier *et al.*^[77] designed a study to evaluate HRV in patients with idiopathic dilated cardiomyopathy to determinate its prognostic value. The group of patients with depressed SDNN (< 100 ms.) had an increased risk of cardiac death or heart transplantation during the follow-up (49.5 ± 35.6 mo).

In patients with mild-to-moderate ventricular dysfunction and NIDCM, a low SDNN, combined with an increased QT dynamicity, has been found to be associated with an increased risk of arrhythmic events^[50]. However, in other studies, no independent association with arrhythmic events has been found^[44].

HRT is another parameter reflecting autonomic control of heart rate. It is the expression of the baroreflex-mediated transient acceleration-deceleration response of the sinus node triggered by a premature ventricular beat (PVB)^[78]. HRT is a baroreflex-mediated biphasic reaction of heart rate in response to premature ventricular beats. It is quantified by: turbulence onset (TO) reflecting the initial acceleration of heart rate following premature beat; and turbulence slope (TS) describing subsequent deceleration of heart rate following a premature ventricular beat. TO is the percentage of relative change in the mean of 2 RR intervals after a PVB. TS is the slope of the steepest regression line computed over the sequence of every 5 consecutive RR intervals following a PVB within 15 RR and is expressed in ms/RR. HRT can be calculated only in patients with sinus rhythm presenting with eligible PVBs^[79]. Abnormal HRT identifies patients with an autonomic dysfunction or impaired baroreflex sensitivity due to a variety of disorders, but may also reflect changes in the autonomic nervous system induced by different therapeutic modalities such as drugs, revascularization or cardiac resynchronization therapy^[80]. HRT has been introduced as an autonomic predictor for cardiac events in heart failure patients and in large cohorts of postinfarction patients^[80-91], as summarized in Table 4. The retrospective analysis of the ATRAMI trial^[81] showed that HRT identified postinfarction patients at risk of both all-cause death and arrhythmic events. Other large trials confirmed the prognostic role of abnormal HRT for predicting mortality and arrhythmic events in postinfarction patients^[85,89] as well as in both NIDCM and IDCM patients^[88,90]. However, the results of the studies, particularly in NIDCM, are conflicting. In the Marburg study, Grimm *et al.*^[84] observed that in 242 patients with idiopathic cardiomyopathy, HRT onset is a significant predictor of transplant-free survival, but for arrhythmia risk stratification, only LVEF remained a significant risk predictor on multivariate analysis. Moreover, analysis of the Frankfurt DCM database showed that HRT and HRV did not yield predictive power for arrhythmic events^[87].

Cardiac denervation assessed by nuclear imaging

In the pathophysiology of malignant ventricular arrhythmias, a relevant role is played not only by sympathetic au-

tonomic nervous system hyperactivity but also by cardiac sympathetic denervation. The presence of cardiac denervation can cause heterogeneity in a refractory period of the ventricular myocardium, thus favoring the onset and the persistence of ventricular arrhythmias. A scintigraphic approach using ¹²³I-labeled metaiodobenzylguanidine (MIBG) can explore the presence of abnormalities in cardiac sympathetic innervation^[92-96].

This radiotracer is administered at rest and planar and single-photon emission computerized tomography images are then acquired after 15 min (early) and 3-5 h (delayed). Generally, the analysis of MIBG distribution is based on the delayed images which reflect overall cardiac sympathetic function, including uptake, re-uptake, storage and release processes of norepinephrine at presynaptic nerve terminals, rather than real time, beat-by-beat sympathetic drive^[96]. The quantitative index calculated after MIBG injection is the heart/mediastinal ratio (H/M). This is derived by the mean counts per pixel of the region of interest drawn over the heart and that drawn over the upper mediastinum^[97]. The value of H/M range is from 1.9 to 2.8 in a normal subject. A normal H/M ratio reflects the density of receptors and the integrity of presynaptic nerve terminals and uptake function. A low H/M ratio reflects a reduced myocardial uptake and a poor cardiac adrenergic receptor density^[95,98].

Besides global myocardial uptake (heart-to-mediastinum ratio), other markers have been used, including washout kinetics and regional uptake heterogeneity. The myocardial washout rate (WR) is expressed as the rate of decrease in myocardial counts over time between early and late imaging, reflecting the neuronal integrity or sympathetic tone^[98]. In HF patients, high myocardial WR and low early and delayed H/M are detectable^[99-101].

The presence of an altered distribution of MIBG can also be found in NIDCM patients^[102] and has been associated with other parameters reflecting arrhythmic risk^[103-104].

Over the last three decades a number of studies have reported the relevance of an altered MIBG distribution in predicting increased risk of death and arrhythmic events^[105-115]. In a group of patients with heart failure, Nakata *et al.*^[101] revealed that impaired cardiac sympathetic innervation assessed by MIBG activity has an incremental and prognostic role for predicting cardiac death and may be useful for identifying a threshold level for selecting patients at risk for death by heart failure, sudden cardiac death and fatal myocardial infarction.

The largest trial evaluating the prognostic role of cardiac denervation assessed by MIBG is the ADMIRE study^[108], in which a total of 961 subjects with NYHA functional class II/III HF and LVEF $\leq 35\%$ were evaluated. Time to first occurrence of NYHA functional class progression, a potentially life-threatening arrhythmic event, and cardiac death were the end-points considered. For H/M < 1.60 , 2 year probabilities of cardiac death and all-cause mortality were 11.2% and 16.1% *vs* 1.8% and 3% for the group with H/M ≥ 1.60 . Moreover, non-fatal arrhythmic events or sudden cardiac death

Table 4 The main studies evaluating the prognostic significance of heart rate turbulence and risk stratification

Ref.	Clinical setting	Number of patients	Cut-off proposed	End-points (mean follow-up)	Results
Schmidt <i>et al</i> ^[80] , 1999	Postinfarction patients	577	TO 0% TS 2.5 ms/RR	All-cause mortality (follow-up 22 mo)	HRT2 predictive for all-cause mortality
Ghuran <i>et al</i> ^[81] , 2002	Postinfarction patients (ATRAMI)	1212	TO 0% TS 2.5 ms/RR	Combined end-point of fatal and non fatal cardiac arrhythmias (follow-up 21 mo)	HRT associated with endpoints
Barthel <i>et al</i> ^[83] , 2003	Postinfarction patients (ISAR-HRT)	1455	TO 0% TS 2.5 ms/RR	All-cause mortality (follow-up 22 mo)	HRT independent predictor of mortality in patients with LVEF $\geq 30\%$
Grimm <i>et al</i> ^[84] , 2003	NIDCM, LVEF $\leq 30\%$	242	TO 0% TS 2.5 ms/RR	Transplant-free survival (follow-up: 41 mo)	TO predictor of transplant-free survival. TO and TS only as univariate predictor of MCE
Exner <i>et al</i> ^[85] , 2007	Myocardial infarction (REFINE)	322	TO 0% TS 2.5 ms/RR	Cardiac death or resuscitated cardiac arrest (follow-up 47 mo)	HRT (10-14 wk after MI) predictive for cardiac death or resuscitated cardiac arrest
Cygankiewicz <i>et al</i> ^[86] , 2008	CHF (IDCM/and NIDCM)	607	TO 0% TS 2.5 ms/RR	All-cause mortality, sudden death and heart failure death (follow-up: 44 mo)	Abnormal TS predictive for all-cause mortality, sudden death and heart failure death
Klingenheben <i>et al</i> ^[87] , 2008	NIDCM (Mean LVEF 28%)	114	TO 0% TS 2.5 ms/RR	Arrhythmic events (follow-up 22 mo)	HRT non predictive for arrhythmic events
Miwa <i>et al</i> ^[88] , 2009	IDCM (241) and NIDCM (134)	375	TO 0% TS 2.5 ms/RR	Cardiac mortality Combined endpoint of cardiac death and/or stable sustained VT (follow-up 15 mo)	Abnormal HRT predictive for cardiac mortality and combined endpoint Prognostic value observed in both ischemic and non-ischemic cardiomyopathy
Huikuri <i>et al</i> ^[89] , 2009	Postinfarction CARISMA	312	TS 2.5 ms/RR	Primary endpoint of documented VT/TV (follow-up 2 yr)	TS evaluated at 6 wk after MI predictive for primary endpoint No prognostic value for HRT evaluated 1 wk after MI
Ikedo <i>et al</i> ^[90] , 2011	NIDC	134	TO 0% TS 2.5 ms/RR	Combined endpoint of cardiac mortality and sustained VT (follow-up 15 mo)	Abnormal HRT predictive for combined endpoint
Miwa <i>et al</i> ^[91] , 2012	IDCM / NIDCM (LVEF $\leq 40\%$)	299	TO 0% TS 2.5 ms/RR	Combined endpoint of sudden cardiac death and sustained VT (follow-up 32 mo)	Abnormal HRT predictive for combined endpoint

HRT: Heart rate turbulence; NIDCM: Non-ischemic dilated cardiomyopathy; TO: Turbulence onset; TS: Turbulence slope; MCE: Major cardiac events; LVEF: Left ventricular ejection fraction; CHF: Chronic heart failure; NYHA ICD: Implantable cardioverter defibrillator.

were observed in patients with $H/M < 1.60$. ADMIRE-HF provided prospective validation of the independent prognostic value of MIBG in the assessment of patients with HF, in identifying patients at high risk of arrhythmic events, sudden cardiac death and ICD discharge.

Finally, it is worth noting that the prognostic significance of MIBG in predicting sudden death has also been demonstrated in a small population of patients with mild-to-moderate CHF^[112].

THE MULTIPARAMETRIC APPROACH TO ARRHYTHMIC RISK STRATIFICATION

Different studies evaluating the role of non-invasive diagnostic tools in predicting arrhythmic events have demonstrated that the combination of the different parameters could be a useful approach in order to better improve arrhythmic risk stratification. Generally, the combination of the different parameters allows the identification of

a smaller group of patients at higher risk of arrhythmic events.

In our series of patients^[49], by combining LVEF ($< 35\%$ *vs* $> 35\%$), NSVT and QTe-slope (> 0.19 *vs* < 0.19), arrhythmic events were more frequently observed in patients with NSVT and a low LVEF and in those with a low LVEF and steeper QTe slope. No significantly higher risk was observed in patients with a higher LVEF and NSVT or steeper QTe slope. When all three variables were considered together, the patients with a low LVEF and NSVT or a steeper QTe slope were found to have a higher arrhythmic risk. In the subgroup of patients with LVEF $< 35\%$, the presence of NSVT and QTe slope > 0.19 defined a small population with the highest probability of events.

Also, among HF patients with a LVEF $> 35\%$, the combination of different arrhythmic risk parameters improved prognostic stratification. Cygankiewicz *et al*^[50] demonstrated that in this population of patients, the presence of two or more independent risk parameters

(SDNN \leq 86 ms, HRT $<$ 2.5 ms/RR and QTc slope $>$ 0.21) detected a population at higher risk of death (30% 3 year mortality) and sudden death (12%), with a rate of events similar to that observed among patients with LVEF \leq 35%.

Merchant *et al.*^[114] tried to assess whether a multi-marker strategy would provide more robust SCD risk stratification than LVEF alone. The authors observed that a multivariable model based on the presence of coronary artery disease, LVEF and MTWA status provides a significantly more robust SCD risk prediction than LVEF as a single risk marker. These findings suggest that multi-marker strategies based on different aspects of the electroanatomic substrate may be capable of improving primary prevention implantable cardioverter-defibrillator treatment algorithms.

Finally, Yukinaka *et al.*^[115] correlated the incidence of ventricular arrhythmias with mismatches in myocardial ^{99m}Tc-methoxyisobutylisonitrile/MIBG accumulation and late ventricular potentials. Patients with late ventricular potentials had greater I-123 MIBG defect scores. The combination of late ventricular potentials and I-123 MIBG uptake could improve the prediction of ventricular arrhythmias after myocardial infarction.

LIMITATIONS OF ALTERNATIVE NON-INVASIVE ARRHYTHMIC RISK PARAMETERS

Although the above mentioned studies have provided evidence about the independent association among a number of parameters and the risk of malignant ventricular arrhythmias, their routine use is still limited for different reasons. In particular, most of the parameters have shown conflicting results, probably related to the methodological differences, such as the studied population (NIDCM or IDCM), the follow-up duration, the end-points considered and the pharmacological treatment at the enrolment. Moreover, all measures are affected by both technical and biological limitations. Finally, almost all these studies were aimed at only evaluating the associations between the studied parameters and the occurrence of ventricular arrhythmias, but not to demonstrate their ability to select patient populations who could benefit from ICD implantation. This ability could be demonstrated only by randomized studies that, to date, are still lacking.

CONCLUSION

Malignant ventricular arrhythmias and sudden death are the main adverse events affecting the prognosis of both NIDCM and IDCM. ICD implantation, *i.e.*, the best therapeutic strategy to reduce the incidence of sudden death, is currently mainly guided by the estimation of LVEF. However, this measure is affected by a number of technical and biological limitations. For these reasons, the best assessment of arrhythmic risk is still a challenge. The use

of other non-invasive parameters reflecting functional or anatomical arrhythmic substrate (LGE), sympathetic nervous activity (HRT, SDNN, the presence of sympathetic denervation by MIBG) and the abnormalities in myocardial refractoriness (QT dynamicity/variability, MTWA) could be useful in order to better characterize both patients with reduced and preserved LVEF at higher risk of arrhythmic events.

Although several studies have shown these parameters to be independently associated with events, their routine use is still limited due to the lack of randomized studies demonstrating their ability to select patient populations who could benefit from ICD implantation. Future prospective studies should aim to reduce this gap in the evidence in order to justify the indication of these techniques in daily clinical practice.

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WJC 6th Anniversary Special Issues (3): Cardiomyopathy

Mechanisms underlying the impaired contractility of diabetic cardiomyopathy

Marie-Louise Ward, David J Crossman

Marie-Louise Ward, David J Crossman, Department of Physiology, Faculty of Medical and Health Sciences, University of Auckland, Auckland 1023, New Zealand

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Correspondence to: Marie-Louise Ward, PhD, Department of Physiology, Faculty of Medical and Health Sciences, University of Auckland, Private Bag 92019, Auckland 1023, New Zealand. m.ward@auckland.ac.nz

Telephone: +64-9-9234889 Fax: +64-9-3737499

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Abstract

Cardiac dysfunction is a well-known consequence of diabetes, with sustained hyperglycaemia leading to the development of a cardiomyopathy that is independent of cardiovascular disease or hypertension. Animal models of diabetes are commonly used to study the pathophysiology of diabetic cardiomyopathy, with the hope that increased knowledge will lead ultimately to better therapeutic strategies being developed. At physiological temperature, left ventricular trabeculae isolated from the streptozotocin rat model of type 1 diabetes showed decreased stress and prolonged relaxation, but with no evidence that decreased contractility was a result of altered myocardial Ca^{2+} handling. Although sarcoplasmic reticulum (SR) Ca^{2+} reuptake appeared slower in diabetic trabeculae, it was offset by an increase in action-potential duration, thereby maintaining SR Ca^{2+} content and favouring increased contraction force. Frequency analysis of t-tubule distribution by confocal imaging of ventricular tissue labeled with wheat germ agglutinin or ryanodine receptor antibodies showed a reduced T-power for diabetic tissue, but the differences were minor in comparison to other models of heart failure.

The contractile dysfunction appeared to be the result of disrupted F-actin in conjunction with the increased type I collagen, with decreased myofilament Ca^{2+} sensitivity contributing to the slowed relaxation.

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Key words: Diabetic cardiomyopathy; Heart failure; Contractility; T-tubules; Excitation-contraction coupling; Calcium homeostasis

Core tip: Diabetic patients develop a cardiomyopathy that is independent of vascular disease, and is thought to develop as a direct result of the prolonged hyperglycaemia. Animal models of diabetes can help us understand the cellular mechanisms that lead ultimately to contractile dysfunction of diabetic cardiomyopathy. The streptozotocin rat model of type 1 diabetes has slowed Ca^{2+} transients and twitch force kinetics, with reduced myofilament Ca^{2+} sensitivity. Myocytes are decreased in volume in diabetic hearts, with reduced and disrupted F-actin, and type 1 collagen is increased. Together, these changes all contribute to the reduced contractility of diabetic cardiomyopathy.

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INTRODUCTION

Patients with diabetes develop a cardiomyopathy that is independent of coronary artery disease and hypertension^[1], and contributes to the increased mortality and morbidity of the disease^[2,3]. The mechanisms that lead to

development of the diabetic cardiomyopathy are poorly understood, although they appear to be a direct result of cellular damage from the hyperglycaemia. The early stages of the cardiomyopathy are associated with reduced diastolic function, with 27%-70% of asymptomatic diabetic patients showing some form of diastolic abnormality^[4-6]. Later this progresses to include systolic dysfunction and heart failure^[7,8]. Diabetes manifests in two forms, both of which are a result of abnormal glucose metabolism. Type I diabetes usually has its onset early in life and is characterized by insufficient insulin production, whereas type II diabetes has its origin downstream of insulin binding to its receptor, and is therefore known as insulin-resistant diabetes. Diabetic cardiomyopathy develops in both type I and type II forms of the disease^[9,10].

Although the heart contains many different cell types, it is the cardiac myocytes that perform the work that enables the heart to function as a pump. With each cardiac cycle, the myocytes experience rapid changes in intracellular ion concentrations that are crucial to the hearts inotropy, lusitropy, and energy metabolism. This review will outline the ultrastructural and functional changes that contribute to the impaired contraction and relaxation characteristic of diabetic cardiomyopathy.

MECHANISMS CONTRIBUTING TO DIABETIC CARDIOMYOPATHY

Streptozotocin rat model of diabetes

Animal models have frequently been used in research into the cellular mechanisms associated with diabetes^[11], with the insulin-deficient streptozotocin rat (STZ) commonly studied. Type-1 diabetes in humans is characterized by the destruction of the pancreatic β -cells, as occurs in the STZ. Streptozotocin is a naturally occurring glucose analog that is particularly toxic to the insulin-producing beta cells of the pancreatic islets. The chemical is transported into cells *via* the glucose transporter-2 (GLUT-2)^[12]. Since the pancreatic beta cells have high levels of GLUT-2, they accumulate streptozotocin in large quantities, resulting in their destruction and the onset of a diabetic state. Rats treated with a single dose of streptozotocin (60 mg/kg) rapidly develop biochemical and functional myocardial abnormalities. They exhibit increased water consumption (180 mL/d compared to 43 mL/d for sham-injected control) and elevated plasma glucose levels (31 mmol/L compared to 4 mmol/L for control) that are sustained. Isolated cardiac muscle preparations from diabetic rats 8 wk post-injection show depressed contractility, diminished compliance and decreased inotropic drug responses^[13]. Abnormalities in contraction and metabolism have been reported both *in vivo* and *in vitro* in the STZ diabetic rat model, reflecting changes at the cardiac myocyte level as a result of the sustained hyperglycaemia. The STZ rat has proved an invaluable model for investigation of the pathogenesis of type 1 diabetes and its complications, and in the development of potential new treatments for the disease^[14-16].

The reduced contractility of diabetic hearts

Contraction in cardiac muscle is brought about by an increase in the myocyte intracellular Ca^{2+} concentration (the “ Ca^{2+} transient”). Propagation of the action potential across the surface sarcolemma and throughout the transverse tubule system (t-tubules) opens voltage-gated L-type Ca^{2+} channels causing a synchronised influx of Ca^{2+} into the myocytes (the “ Ca^{2+} current”). This Ca^{2+} current then triggers release of Ca^{2+} from the junctional region of the sarcoplasmic reticulum (SR) *via* the ryanodine receptors (RyRs) in a process termed “ Ca^{2+} -induced Ca^{2+} -release”^[17,18]. In this way the intracellular Ca^{2+} concentration $[\text{Ca}^{2+}]_i$ is rapidly increased to approximately 10 times the resting level. Ca^{2+} then diffuses to the contractile proteins where it binds to troponin C, initiating cross-bridge cycling and force development. Excitation-contraction coupling has therefore been a major focus of those investigating the cellular mechanisms that underlie the reduced contractility of failing hearts.

Intracellular calcium transients in diabetic hearts

Measurements carried out on multicellular trabeculae isolated from the left ventricle under near physiological conditions (1.5 mmol/L $[\text{Ca}^{2+}]_o$, 37 °C and 5 Hz) showed trabeculae from diabetic rats had depressed contractility with prolonged contraction and relaxation in comparison to their controls, consistent with other studies^[19-21].

An alteration of intracellular Ca^{2+} homeostasis has previously been suggested as underlying the diabetic cardiac dysfunction (for review see^[22]) although, as noted, results are often contradictory. While some of these discrepancies might be attributable to the extent of disease progression (diabetic stage) and experimental conditions, very few studies have examined the $[\text{Ca}^{2+}]_i$ control of contractility under near-physiological temperatures and rates of stimulation. Our study showed that diabetic rats had an unchanged resting $[\text{Ca}^{2+}]_i$ level and amplitude of Ca^{2+} transient, despite a reduced contractility^[23]. Averaged Ca^{2+} transients and isometric twitches at 5 Hz stimulation are shown in Figure 1 for trabeculae from control (solid line) and diabetic (dotted line) rats, superimposed for comparison. Figure 1C shows the $[\text{Ca}^{2+}]_i$ -stress phase plot, with a right shifted relaxation phase for diabetic trabeculae which suggests diminished myofibrillar Ca^{2+} sensitivity.

Figure 2 shows averaged data from trabeculae at 5 Hz stimulation and at 37 °C. Diabetic rats had prolonged time-to-peak $[\text{Ca}^{2+}]_i$ and a prolonged time constant of Ca^{2+} transient decay, consistent with some other reports^[20,24-26]. The slower kinetics of Ca^{2+} transient would contribute to the prolonged time course of cardiac contraction and relaxation in diabetic rats, but it is unclear if the reduced rate of the decay in the Ca^{2+} transient is sufficient to explain the slowed mechanical relaxation.

Our study showed that contractility was reduced in trabeculae from diabetic hearts, even when peak $[\text{Ca}^{2+}]_i$ was matched between diabetic and control trabeculae by altering stimulation rate^[23], suggesting that altered $[\text{Ca}^{2+}]_i$ handling was not the primary mechanism of contractile

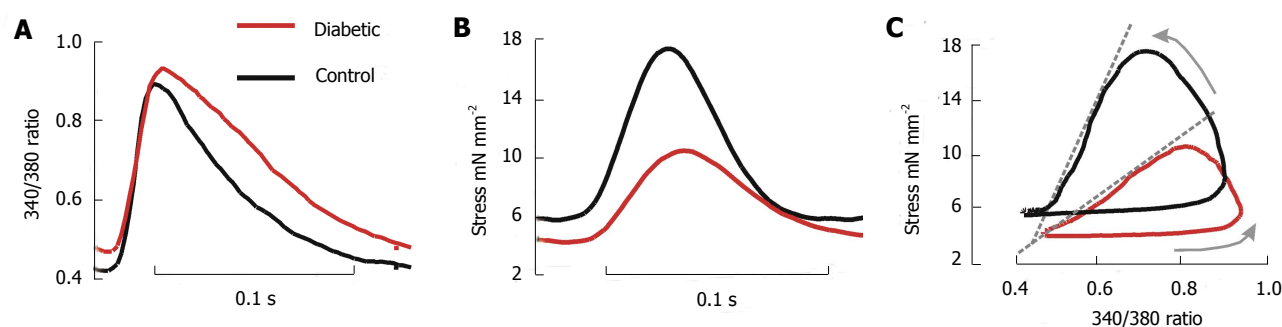


Figure 1 Average intracellular Ca^{2+} transients and isometric stress. Data were recorded from left ventricular trabeculae of diabetic (red lines) and control (black lines) hearts at 5 Hz, 37 °C, and 1.5 mmol $[\text{Ca}^{2+}]_o$, 7 trabeculae per group. A: Ca^{2+} transient (340/380 fluorescence ratio); B: Stress; C: Phase plots of the relationship between fluorescence and stress. The arrows indicate the direction of time, and the dashed grey lines accentuate the slope of the relaxation component. (Modified from Zhang *et al*^[23]).

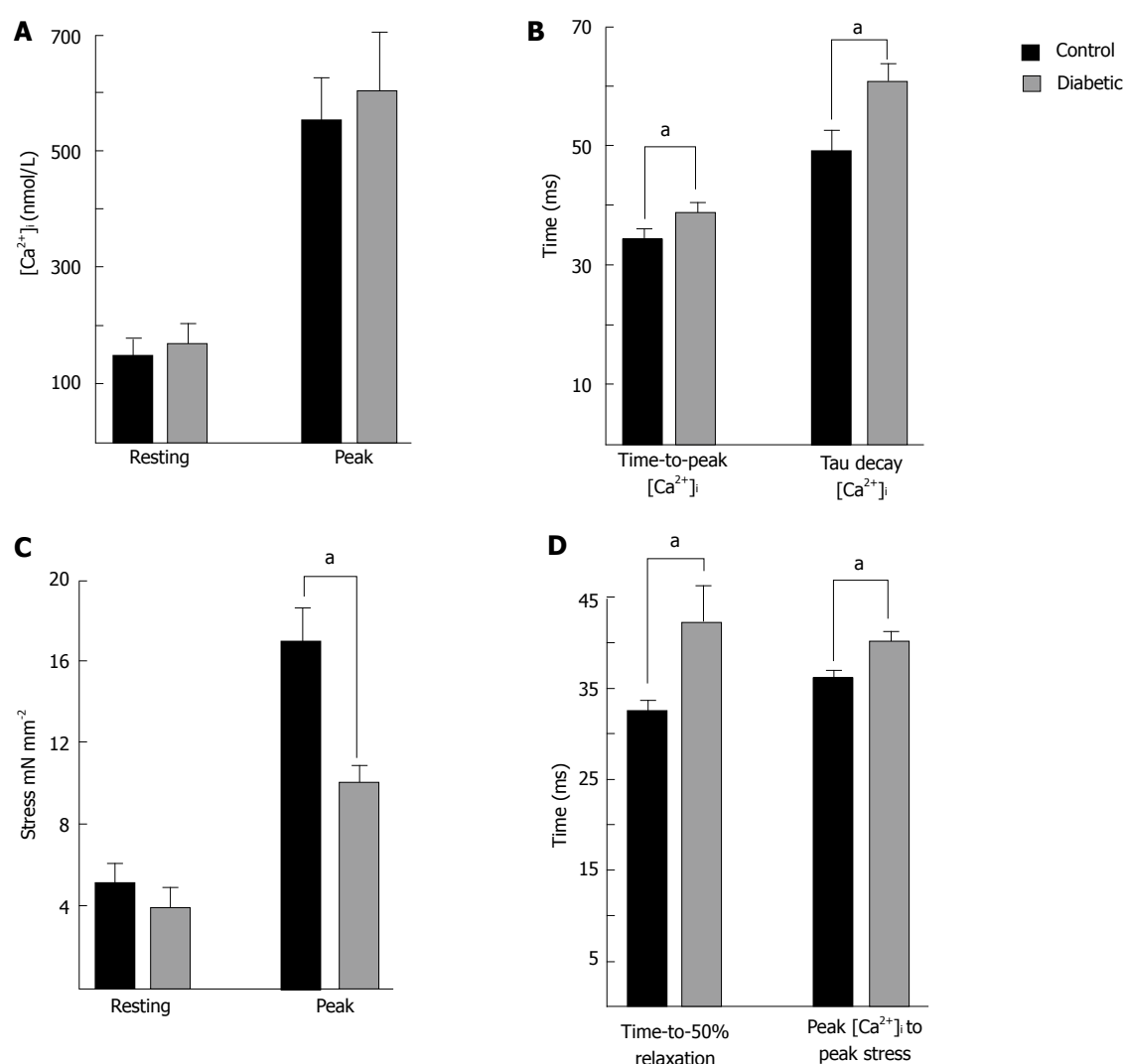


Figure 2 Summary of intracellular Ca^{2+} and isometric stress parameters. Data were recorded from left ventricular trabeculae at 37 °C, 5 Hz stimulation frequency, and 1.5 mmol $[\text{Ca}^{2+}]_o$. Data are mean \pm SE 8 wk post injection for control ($n = 7$) and diabetic ($n = 8$). A: Shows resting and peak $[\text{Ca}^{2+}]_i$. The Ca^{2+} transients were prolonged in diabetic trabeculae; B: Shows the time to reach peak $[\text{Ca}^{2+}]_i$, and the time constant of the Ca^{2+} transient decay; C: Shows no difference in resting stress, but peak stress was reduced in diabetic trabeculae; D: Shows the time to 50% relaxation of stress was prolonged in diabetic, as was the time from the peak of the Ca^{2+} transient to the peak of the twitch. * $P < 0.05$, diabetic vs control.

dysfunction. The mechanical relaxation was intrinsically slower in diabetic rat hearts, which was exacerbated by the reduced rate of decrease of $[\text{Ca}^{2+}]_i$. In support of this

idea, Figure 2D shows that the interval between the time-to-peak $[\text{Ca}^{2+}]_i$ and the time-to-peak stress in diabetic rats was increased in comparison to control.

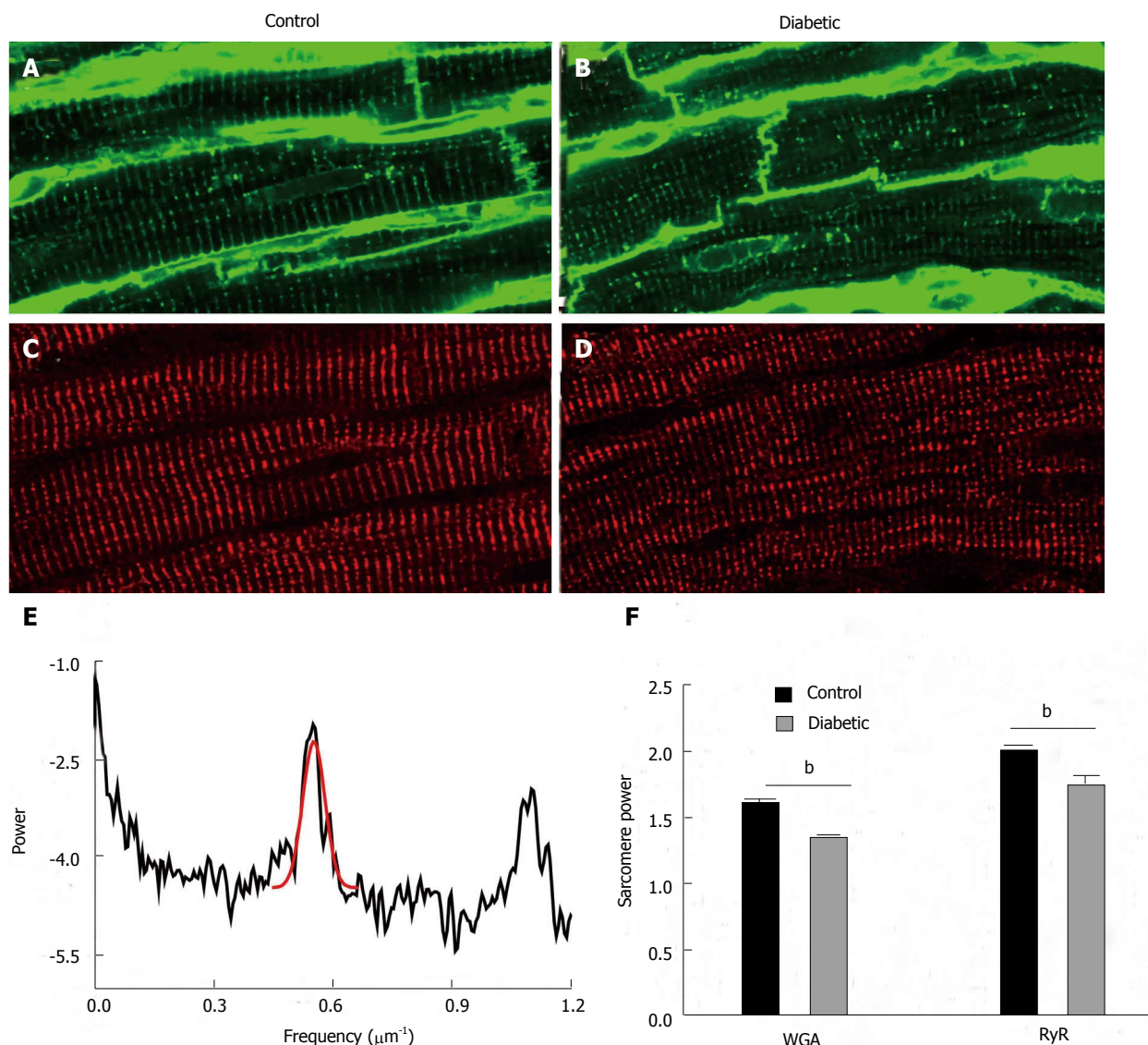


Figure 3 Structural changes in proteins associated with excitation-contraction coupling. Transverse tubules were visualised by labelling with wheat germ agglutinin in (A) control and (B) diabetic tissue. The same tissue sections were dual labelled with antibodies against ryanodine receptors (RyR) in (C) control and (D) diabetic tissue. The periodicity or regularity of labelling was assessed using a fast Fourier transform. An example of this analysis is shown in (E) which is the plot of the FFT in control myocyte labelled with RyR. The peak associated with sarcomeric periodicity (approximately $0.55 \mu\text{m}^{-1}$) is fitted with a Gaussian in red. The height of this peak is used as a metric to assess the regularity of sarcomere labelling termed "sarcomere" power. (F) This shows the mean sarcomere power for both wheat germ agglutinin and ryanodine receptor labelling from 18 cells from 3 control animals and 18 cells from 3 diabetic animals. Both wheat germ agglutinin and ryanodine receptor sarcomere power were modestly but highly significantly reduced in cells from diabetic hearts (Bonferroni corrected *t* test, $^bP < 0.01$, diabetic vs control.).

Analysis of electrocardiogram (ECG) in lightly anesthetized diabetic rats prior to experimentation showed that the normalized QT interval was prolonged, implying the cardiac action potential was slower^[23]. This would contribute to the prolonged Ca^{2+} transients observed in diabetes, but cannot explain the observed Ca^{2+} transient changes in full. Logarithmic plots of Ca^{2+} transients from control and diabetic trabeculae in Zhang *et al*^[23] (2008) show that the linear portion of the Ca^{2+} fluorescence decay was delayed in trabeculae from diabetic hearts, consistent with the increase in the time-to-50% repolarization of the ventricular action potential reported in their study. Prolonged depolarization during the plateau phase of the action potential will lead also to increased L-type Ca^{2+} influx, although this was not shown in the Zhang *et al*^[23]

(2008) study. Frequently studies have reported changes in SERCA protein expression in explanation of observed changes to the time course of the Ca^{2+} transients^[27,28], but decreased SERCA activity and/or expression may only contribute in part to the prolonged Ca^{2+} transient decay. Action potential duration is also important in determining the duration of the Ca^{2+} transient, and therefore the SR Ca^{2+} load, which in turn determines SR Ca^{2+} release *via* the RyRs^[29]. ECG measurements in insulin-treated type 1 diabetic patients also show abnormal repolarization with the reports of increased QT interval and increased QT dispersion^[30].

T-tubule system structure

The t-tubules are an important component of the excita-

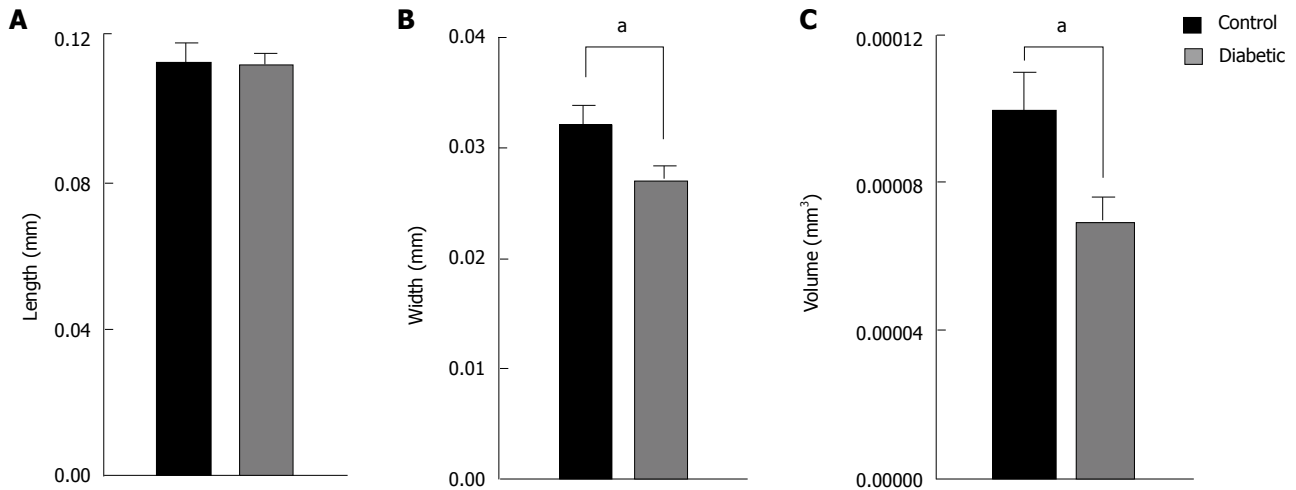


Figure 4 Average dimensions of isolated ventricular myocytes from diabetic and control rat hearts. Cell length (A) was not different between diabetic ($n = 35$) and control ($n = 19$) hearts, whereas cell width (B) and cell volume (C) was reduced. ^a $P < 0.05$, diabetic vs control.

tion-contraction coupling system in cardiac myocytes^[31]. T-tubules are an extension of the sarcolemma that project transversely into the interior of the cell adjacent to the z-line, although numerous axial connections between sarcomeres are observed^[32]. This structure facilitates synchronous contraction by conducting the action potential deep within the myocyte and triggering Ca^{2+} release from the SR in regions located away from the cell surface. There is evidence that loss of normal transverse tubule structure is a key feature of both animal^[33,34] and human heart failure^[35,36]. Frequency analysis of t-tubule distribution at the z-line has been used to quantify the structural changes in t-system labelling of myocytes from rodents at different stages of heart failure^[33]. This analysis exploits the periodic nature of t-tubule distribution at the z-line of sarcomere. By converting t-tubule images into the frequency domain with a fast Fourier transform, a peak associated with sarcomere spacing of $2\ \mu\text{m}$ is observed in the power spectrum^[37,38]. In failing myocytes the periodic pattern of t-tubule labelling is disrupted resulting in reduced sarcomere peak. This peak is termed “T-power” and provides a useful metric to quantify t-tubule structure.

Currently there is lack of comparable data for changes in t-tubules in the diabetic heart. To address this gap in knowledge we have used confocal laser scanning microscopy to examine the labelling of the t-tubules [wheat germ agglutinin (WGA)] and the ryanodine receptors (RyR), in the hearts of STZ rats with end stage heart failure as shown in Figure 3. Analysis of this labelling in the frequency domain has shown a significant but surprisingly modest decrease in T-power (or sarcomere power) in the t-tubule system of diabetic myocytes. A similar analysis of RyR labelling showed a comparable decrease in sarcomere power in diabetic myocytes. Visual inspection of the labelling in Figure 3 shows that both the structure of t-system (WGA) and the SR (RyR) are largely intact in myocytes from diabetic rat hearts, which is consistent with the comparatively normal calcium transients measured in the cardiac trabeculae of this animal

model^[23]. This contrasts with the dramatic loss of the t-system structure reported in non-diabetic animal heart failure. For example the t-system is dramatically remodelled in spontaneously hypertensive rat, while the labelling of RyR is largely intact^[34,38]. A similar situation is seen in non-diabetic human heart failure^[36]. This may turn out to be a key point of difference between diabetic and other forms of heart failure, and it remains to be seen if a similar pattern of t-system preservation is seen in the diabetic human heart. Alternatively, the lack of obvious changes in the t-tubule distribution of STZ-induced diabetic rat hearts 8 wk post injection may reflect the relatively short duration of the disease. Figure 2B shows an increase in the time-to-peak of the Ca^{2+} transients in longitudinal section (LV) trabeculae from diabetic hearts, which may reflect changes in excitation-contraction coupling from hyperglycaemia-induced loss of t-tubule structure.

Ventricular remodeling of diabetic hearts

Although intracellular Ca^{2+} cycling is essential to the contraction and relaxation of cardiac myocytes, the extracellular matrix and the myofilaments within the myocytes are essential also. The contractile proteins that make up the myofilaments are the end effectors of excitation-contraction coupling, and their responsiveness to Ca^{2+} directly determines myocyte contractility (for reviews see^[39,40]). Changes in the contractile proteins of diabetic hearts have been reported, and are likely to contribute substantially to the observed changes in contraction and relaxation. Figure 2C and D show both reduced contraction (peak stress) and slowed relaxation in LV trabeculae from diabetic rat hearts. The slower time course of contraction in trabeculae from diabetic hearts could be explained, in part, by a shift in the myosin isoenzyme distribution from the faster alpha heavy chain to the beta form as previously reported^[41] (for review see^[42]). Changes in other aspects of the contractile protein system have also been described in diabetic hearts. The thin filament regulatory troponin-tropomyosin complex shows decreased Ca^{2+} sensitivity in skinned^[43,44] and intact^[16] cardiac muscle

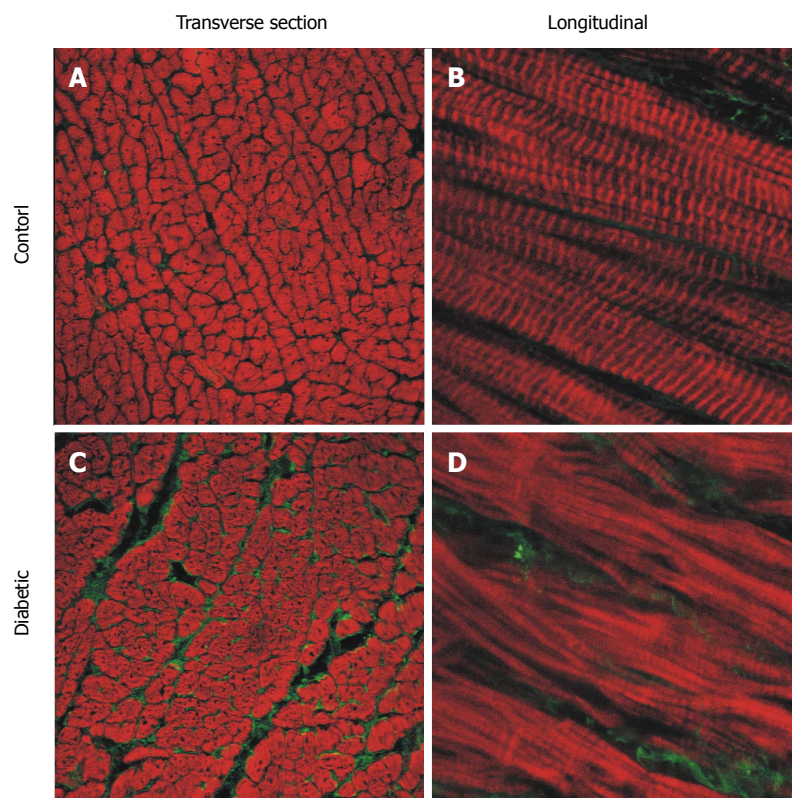


Figure 5 Representative confocal images of longitudinal section free wall immuno-labelled for type I collagen (green) and f-actin (red). Sections from the endocardium of control (A and B) and diabetic (C and D) rat hearts. Left hand side panels: Transverse sections from endocardium (25 × objective). Right hand side panels: Longitudinal sections (63 × objective, zoom × 3). (Modified from Zhang *et al.*^[23]).

preparations. The consequence of reduced Ca^{2+} sensitivity is increased force production for any given cytosolic Ca^{2+} concentration, favouring force production during systole, but decreasing relaxation which would contribute to diastolic failure.

Ultra-structural analysis by electron microscopy has revealed loss and disorganisation of actin filaments in STZ diabetic hearts^[21], which was supported by confocal analysis of phalloidin labelled ventricular tissue with disorganisation and a reduction of f-actin labelling evident^[16,21,23]. We have also observed that myocyte cell diameter is reduced in the STZ diabetic rat, suggesting that amount of myofilament protein per myocyte is reduced. Figure 4 shows mean \pm SE data from enzymatically isolated ventricular myocytes from diabetic and control rats. Cell length was not different between groups, but both width and volume were markedly reduced in myocytes from diabetic hearts. Similar changes in f-actin content and myocyte size in the STZ diabetic rat have been reported by Kawaguchi *et al.*^[45] (1999). Pertinently these authors also identified a decrease in myocyte diameter in the diabetic human heart^[45]. Changes in myocyte volume have been shown to occur as early as one week after induction of diabetes in the STZ^[46]. The decreased myocyte volume is evident in the left hand side (LHS) panel of Figure 5C and D where representative tissue from the LV free wall of diabetic rat hearts shows reduced myocyte diameter. It appears then that the diabetic myocytes are atrophied. Ultrastructural changes in mitochondrial morphology have been shown by electron microscopy of diabetic rat heart, with likely consequences not only for myocyte volume but also energy metabolism^[21]. Proteomic analysis of diabetic rat heart identified multitude

of changes in the mitochondrial proteome^[47]. The most notable changes are an increase in enzymes involved in long chain fatty acids oxidation and decrease in enzymes involved in catabolism. Metabolism of the diabetic heart is shifted from a mix of carbohydrates and fatty acids for energy supply to relying almost solely on fatty acids, with a resultant increase in the production of oxygen free radical end products^[48]. Significantly the proteomic analysis also showed changes in proteins involved with oxidative stress, suggesting that impaired energy metabolism might lead to myocytes being unable to meet the energetic needs producing changes in the structure and function of the contractile machinery.

Diabetic cardiomyopathy is also associated with increased stiffness in the left ventricle^[49], and a decreased maximum rate-of-rise in developed stress^[23], suggesting that cardiac compliance is reduced in diabetic rats. The extracellular matrix in healthy hearts provides a scaffolding that supports the myocytes and other tissue components, enabling the coordinated transduction of force that is necessary for the heart to function as a pump. Collagen is an important component of the extracellular matrix, with type I and type III collagens the most abundant types in ventricular tissue forming 90% of the total collagen content^[50]. Figure 5 shows type I collagen is increased in diabetic rat hearts, which would contribute to the decreased ventricular compliance, with no change in type III collagen^[23]. Myocardial echodensity has been reported as increased in asymptomatic diabetic patients, thought to be a result of increased collagen deposition^[51]. It is proposed that increased echodensity might therefore act as an early indicator of the subsequent development of diabetic cardiomyopathy.

CONCLUSION

In conclusion, diabetic cardiomyopathy arises as a result of the sustained hyperglycaemia and the damaging effects this has on the heart. Ventricular myocytes from untreated diabetic rat hearts show contractile dysfunction after 8 wk of hyperglycaemia, with prolonged action potential duration, slower Ca^{2+} transient decay and reduced myofilament Ca^{2+} sensitivity. Gross structural changes to the myocardium are evident at this stage of the disease. Extracellular type 1 collagen is increased, t-tubules are less regular in appearance, and F-actin within myocytes is reduced in content and disrupted in appearance. We conclude that it is these structural changes that are the main contributors to the contractile dysfunction of diabetic cardiomyopathy, along with mitochondrial changes that compromise energy supply. We suggest that consideration should therefore be given in future studies to the contribution of these observed structural changes to the contractile deficit in the diabetic hearts, rather than focusing on myocyte Ca^{2+} handling in searching for effective treatments for diabetic cardiomyopathy.

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WJC 6th Anniversary Special Issues (3): Cardiomyopathy

Distribution of late gadolinium enhancement in various types of cardiomyopathies: Significance in differential diagnosis, clinical features and prognosis

Hiroshi Satoh, Makoto Sano, Kenichiro Suwa, Takeji Saitoh, Mamoru Nobuhara, Masao Saotome, Tsuyoshi Urushida, Hideki Katoh, Hideharu Hayashi

Hiroshi Satoh, Makoto Sano, Kenichiro Suwa, Takeji Saitoh, Mamoru Nobuhara, Masao Saotome, Tsuyoshi Urushida, Hideki Katoh, Hideharu Hayashi, Division of Cardiology, Internal Medicine III, Hamamatsu University School of Medicine, Hamamatsu 431-3192, Japan

Author contributions: All authors contributed to this paper.

Correspondence to: Hiroshi Satoh, MD, PhD, Division of Cardiology, Internal Medicine III, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ward, Hamamatsu 431-3192, Japan. satoh36@hama-med.ac.jp

Telephone: +81-53-4352267 Fax: +81-53-4342910

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Abstract

The recent development of cardiac magnetic resonance (CMR) techniques has allowed detailed analyses of cardiac function and tissue characterization with high spatial resolution. We review characteristic CMR features in ischemic and non-ischemic cardiomyopathies (ICM and NICM), especially in terms of the location and distribution of late gadolinium enhancement (LGE). CMR in ICM shows segmental wall motion abnormalities or wall thinning in a particular coronary arterial territory, and the subendocardial or transmural LGE. LGE in NICM generally does not correspond to any particular coronary artery distribution and is located mostly in the mid-wall to subepicardial layer. The analysis of LGE distribution is valuable to differentiate NICM with diffusely impaired systolic function, including dilated cardiomyopathy, end-stage hypertrophic cardiomyopathy (HCM), cardiac sarcoidosis, and myocarditis, and those with diffuse left ventricular (LV) hypertrophy including HCM, cardiac amyloidosis and Anderson-Fabry disease. A transient low signal intensity LGE in regions of severe

LV dysfunction is a particular feature of stress cardiomyopathy. In arrhythmogenic right ventricular cardiomyopathy/dysplasia, an enhancement of right ventricular (RV) wall with functional and morphological changes of RV becomes apparent. Finally, the analyses of LGE distribution have potentials to predict cardiac outcomes and response to treatments.

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Key words: Cardiomyopathy; Cardiac magnetic resonance; Late gadolinium enhancement; Cardiac function; Clinical features; Prognosis

Core tip: We review characteristic cardiac magnetic resonance (CMR) features in ischemic and non-ischemic cardiomyopathies (NICM), especially in terms of location and distribution of late gadolinium enhancement (LGE). LGE in NICM does not correspond to any particular coronary artery distribution and is located mostly in the mid-wall to subepicardial layer. The analysis of LGE distribution is valuable to differentiate NICM with diffusely impaired systolic function; dilated cardiomyopathy, end-stage hypertrophic cardiomyopathy (HCM), cardiac sarcoidosis, and myocarditis, and those with diffuse LV hypertrophy; HCM, cardiac amyloidosis and Anderson-Fabry disease. The analyses of LGE distribution have potentials to predict cardiac outcomes and response to treatments.

Satoh H, Sano M, Suwa K, Saitoh T, Nobuhara M, Saotome M, Urushida T, Katoh H, Hayashi H. Distribution of late gadolinium enhancement in various types of cardiomyopathies: Significance in differential diagnosis, clinical features and prognosis. *World J Cardiol* 2014; 6(7): 585-601 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i7/585.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i7.585>

INTRODUCTION

The management of patients with left ventricular (LV) dysfunction starts from the identification of underlying myocardial disorders. The primary diagnostic issue is the differentiation between ischemic and non-ischemic cardiomyopathies (ICM and NICM). NICM include several disorders, such as dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), cardiac sarcoidosis, stress cardiomyopathy, and others^[1,2], but often show similar clinical presentations which lead to progressive heart failure, a high risk of fatal arrhythmias, and a high mortality rate^[3].

NICM have been traditionally diagnosed non-invasively with chest roentgenography, standard 12-lead electrocardiography (ECG), transthoracic and/or transesophageal echocardiography and nuclear imaging, and invasively with coronary angiography, left ventriculography, and endomyocardial biopsy.

Imaging with cardiac magnetic resonance (CMR) is non-invasive, uses no ionizing radiation, and has high spatial resolution. Recent advantage of CMR has enabled us to assess cardiac morphology, function and tissue characteristics both in ICM and NICM^[4,5]. Thus, CMR is capable of identifying cardiac abnormalities not readily recognized by conventional imaging modalities^[6-8].

We have been studying the late gadolinium enhancement (LGE) in various NICM and attempting to verify the values for differential diagnosis, clinical features, and prognosis^[9-13]. This review article focuses on various types of NICM, and discusses initially about CMR techniques and differential diagnosis from ICM, and then about the usefulness of CMR, especially the clinical significance of location and distribution of LGE.

RECENT DEVELOPMENT OF CMR

CMR imaging comprises several techniques of magnetic resonance imaging (MRI) sequences. Cine-CMR, which is based on the steady state free precession sequence, provides accurate information about cardiac morphology and function. First-pass contrast enhanced perfusion-CMR with and without vasodilators can provide assessment of myocardial perfusion reserve^[14].

LGE-CMR relies on the delivery of intravenous gadolinium chelate to the myocardium, which is a biologically inert tracer that freely distributes in extracellular space but does not cross the intact cell membrane. Due to a combination of increased extracellular volume and slower washout kinetics, there is a relative accumulation of gadolinium in areas of necrosis, fibrosis, infiltration, and inflammation in the late washout phase. Since gadolinium shortens T1 relaxation time, it produces brighter signal intensity, and this technique is sensitive and reproducible in the detection of myocardial scarring both in

ICM and NICM^[15,16]. However, since LGE is ascribed to relative accumulation of gadolinium in areas of damaged myocardium, LGE-CMR techniques may miss a diffuse type of fibrosis^[16,17]. Recently, T1 mapping with a Look-Locker sequence after injection of gadolinium has become a promising tool to quantify interstitial myocardial fibrosis^[18].

There are also special sequences that are used less often to clarify the cause of NICM. These include fat suppression black blood for detection of fatty infiltration, T2-weighted imaging for myocardial edema, and T2-star (T2*) for the assessment of myocardial iron^[19-21].

Thus, the combination of multiple CMR sequences helps clinicians differentially diagnose NICM. The characteristic features in each CMR sequence will be discussed below under each specific NICM.

DIFFERENTIAL DIAGNOSIS OF ICM AND NICM

The diagnosis of patients with NICM originates with the differentiation of ICM. In general, coronary angiography is routinely performed for the differentiation, and when patients have no obstructive coronary arteries or coronary risk factors, the diagnosis of NICM is usually made. However, it has to be kept in mind that no obstructive coronary artery on angiography is inadequate to exclude ICM^[16]. The spontaneous recanalization after coronary occlusion caused by a rupture of minimally stenotic but unstable plaque, embolization or spasm may mask the occurrence of coronary events. Conversely, it is also a common situation that patients with DCM have coronary arterial disease during their natural courses. An autopsy study in some patients diagnosed with DCM has described subendocardial and transmural fibrosis indistinguishable from myocardial infarction^[22].

CMR technique is now recognized as a useful tool to determine whether the LV dysfunction is caused by ischemic coronary events. Cine-CMR with excellent spatial and temporal resolution can detect segmental wall motion abnormalities or wall thinning in a particular coronary arterial territory. LGE-CMR can also define the subendocardial or transmural LGE as fibrosis caused by coronary events because the ischemic wave front starts from subendocardium.

On the other hand, LGE in NICM generally does not correspond to any particular coronary artery distribution and is often located in the mid-wall^[23]. A previous study detected striated or patchy pattern of LGE in a certain part of patients diagnosed with DCM^[16].

The differential diagnosis of ICM and NICM is also crucial for management of patients with cardiac dysfunction. Treatment with β -adrenoceptor blockers and renin-angiotensin-aldosterone inhibitors are recommended for both ICM and NICM. Patients with ICM have worse outcome but may benefit from revascularization and/or aneurysmectomy and from secondary prevention with aspirin and statins. Furthermore, LV remodeling after

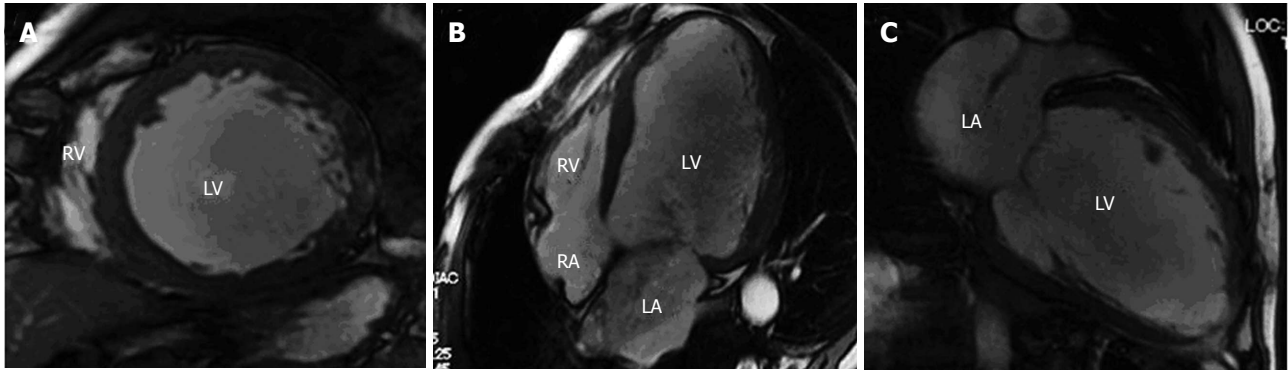


Figure 1 Representative cine-cardiac magnetic resonance images in a 62-year-old male patient with dilated cardiomyopathy. The images show mid-ventricular short axis (A), horizontal axis (4-chambers) (B) and vertical long axis views (C). The images reveal dilatation of left ventricular (LV) cavity and diffuse wall thinning (relatively homogenous). The LV end-diastolic volume, LV end-systolic volume, LV ejection fraction (EF) and LV mass are 329.1 mL, 252.5 mL, and 23.3%, 153.2 g, respectively. LV and RV: Left and right ventricles; LA and RA: Left and right atria; LV: Left ventricular.

myocardial infarction often occurs with non-extensive infarction but the absence of suitable preventive therapy. Conversely, in patients with NICM, the early diagnosis may recommend genetic studies to indentify inherited abnormalities and help to start early aggressive study with intensified medical and device therapies^[2,16].

DCM

General

DCM is the most common isoform of NICM, and is characterized by dilatation of LV chamber and systolic dysfunction, which leads to progressive heart failure, high risk for fatal arrhythmias and high mortality rate^[3].

Although over the half of cases are idiopathic, DCM is not a single tree of disease spectrum but may include several undetermined etiologies, such as chronic myocarditis, tachycardia-induced cardiomyopathy, undiagnosed sarcoidosis, and end-stage HCM^[16,24].

CMR features

In cine-CMR, all cardiac chambers are enlarged and a decrease in LV ejection fraction (EF) is evident. The LV wall thickness is normal or decreased, but relatively homogenous. Figure 1 shows representative cine-CMR images of different views in a patient with DCM.

In LGE-CMR, DCM has been shown to demonstrate mostly a lack of LGE or the presence of mid-wall enhancement, and a fewer part of cases shows patchy or diffuse striated LGE. The distribution of LGE is unrelated to a particular coronary arterial territory, and corresponds to focal fibrosis at autopsy^[1,9,25]. Our recent study showed various patterns of LGE as described in Figure 2^[13]. However, the prevalence of LGE varies among reports between 12% and 67%, which may be caused by different etiologies, disease states and duration, or by a limitation of LGE-CMR technique. The mechanisms of myocardial fibrosis in DCM are complex and include inflammation, genetic predisposition, micro-vascular ischemia, and neurohumoral changes^[9]. LGE-CMR technique may miss a diffuse type of fibrosis, and hence a certain

part of DCM patients may have no LGE^[16,17]. Different thresholds used to detect LGE may also affect the variation in the prevalence of LGE. A recent development of T1 mapping technique is expected to estimate such a diffuse type of fibrosis^[17,18].

Clinical implications

Several previous studies showed the lack of relationship between the presence of LGE or LGE volume, and LV volume and function^[9,12,26]. We and other investigators found that LGE volume did not correlate with LV end-diastolic volume, global left ventricular ejection fraction (LVEF) or segmental LV contraction, but the washout rate of 99m-technecium-sestamibi (^{99m}Tc-MIBI) did^[12,27]. Since the increase in washout rate of ^{99m}Tc-MIBI reflects mitochondrial dysfunction in cardiomyocytes^[28], the increased LV volume and impairment of LV function in DCM may be ascribed to the dysfunction of individual myocytes rather than segmental fibrosis. However, recent studies have shown the resistance of patients with mid-wall LGE to reverse remodeling by β -adrenergic blockers and/or cardiac re-synchronization therapy^[9,29]. We also showed that reverse remodeling occurred after treatment in patients with no LGE and with LGE localized in inter-ventricular septum, but did not in patients with extensively distributed LGE^[13]. Since LV segments with a lower amount of LGE are expected to have more viable but functionally disturbed cardiomyocytes and reversible matrix fibrosis, they are more likely to benefit from therapies^[12,27].

The mid-wall LGE in DCM correlates with intra-ventricular conduction disturbance, and is independently predictive of sudden cardiac death (SCD) or ventricular tachycardias (VTs)^[13,30,31]. Thus, LGE-CMR can help to identify the arrhythmogenic substrate and plan an appropriate mapping and ablation strategy.

In DCM, a series of factors is associated with adverse prognosis, such as age, gender, LVEF, QRS duration and cardiac biomarkers^[13]. Although the larger LGE volume is associated with poor prognosis in patients with ICM^[13,32], the prognostic implication of LGE in DCM remains controversial. However, the severity of irrevers-

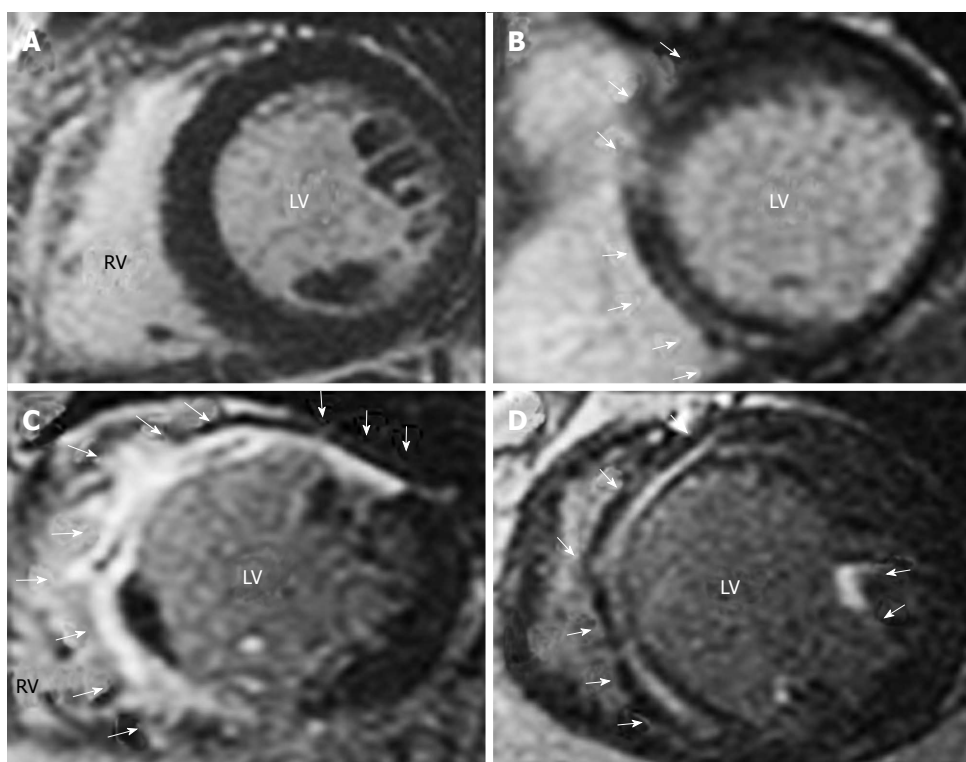


Figure 2 Representative short axis late gadolinium enhancement-cardiac magnetic resonance images in patients with dilated cardiomyopathy. A: No LGE; B: localized LGE. Mid-wall LGE distributed only into anterior and inferior septum; C: Extensive LGE. LGE distributed at anterior and inferior septum, anterior, antero-lateral and inferior LV segments; D: Extensive LGE. Mid wall LGE distributed at anterior and inferior septum, and at anterior papillary muscle. Arrows indicate LGE in LV wall segments. All the images are taken from Machii *et al*^[13] with permission. LGE: Late gadolinium enhancement; LV: Left ventricular.

ible fibrosis is related to the impairment of cardiac function, the propensity to ventricular arrhythmias and the resistance to reverse remodeling, and recent studies have shown that LGE volume is well concordant with high probabilities of cardiac mortality and morbidity^[30,33,34]. We also exhibited the lowest event-free survival rate in patients with extensively distributed LGE^[13]. Therefore, the analysis of LGE volume or distribution, not only the presence of LGE, may be valuable to predict prognosis and identify high-risk patients in DCM.

HCM

General

HCM is a relatively common genetic disorder of the cardiac sarcomere, characterized by an idiopathic LV hypertrophy. Typically, this disorder demonstrates asymmetric septal hypertrophy, but can also present atypical patterns of hypertrophy involving the mid-ventricle and apex. Hence, HCM has a wide variety of morphological, functional, and clinical features.

CMR features

Because of various phenotypic expressions of HCM and other mimicking diseases which show LV hypertrophy, cardiac imaging has a central role in establishing the final diagnosis. Although transthoracic echocardiography has been the standard tool for the diagnosis of HCM, it has limitations for precise visualization of whole ventricles

and quantification of hypertrophy. CMR is capable of identifying regions of LV hypertrophy not readily recognized by echocardiography^[6-8], especially for apical hypertrophy and apical aneurysm^[11,35,36].

The myocardial LGE is a common feature of HCM, and can be focal or spread diffusely into any areas of LV^[11,37,38]. A previous study showed that more than 55% of HCM patients have some LGE, most commonly at the anterior and posterior RV insertion points. Gene-positive patients are more likely to have LGE and may even precede hypertrophy^[39,40]. LGE in HCM usually represents areas of increased interstitial fibrosis but may also indicate myocardial disarray, necrosis, and scarring^[41]. Figure 3 shows representative cine-CMR and LGE-CMR images in various types of HCM.

In other MRI sequences, a previous study showed focal T2 abnormalities in the areas of LGE with severe LV hypertrophy^[42]. In addition, stress CMR can demonstrate reduced vasodilator response in subendocardium particularly in the area of severe hypertrophy^[14].

Clinical implications

In contrast to DCM, the presence of LGE and LGE volume have been well associated with New York Heart Association (NYHA) functional classes, LV systolic and diastolic function, and left atrial volume^[9,11,43]. Since 15% to 20% of HCM patients have progressive heart failure^[44], determining the prognostic implications of LGE in HCM patients is crucial in order to identify high-risk

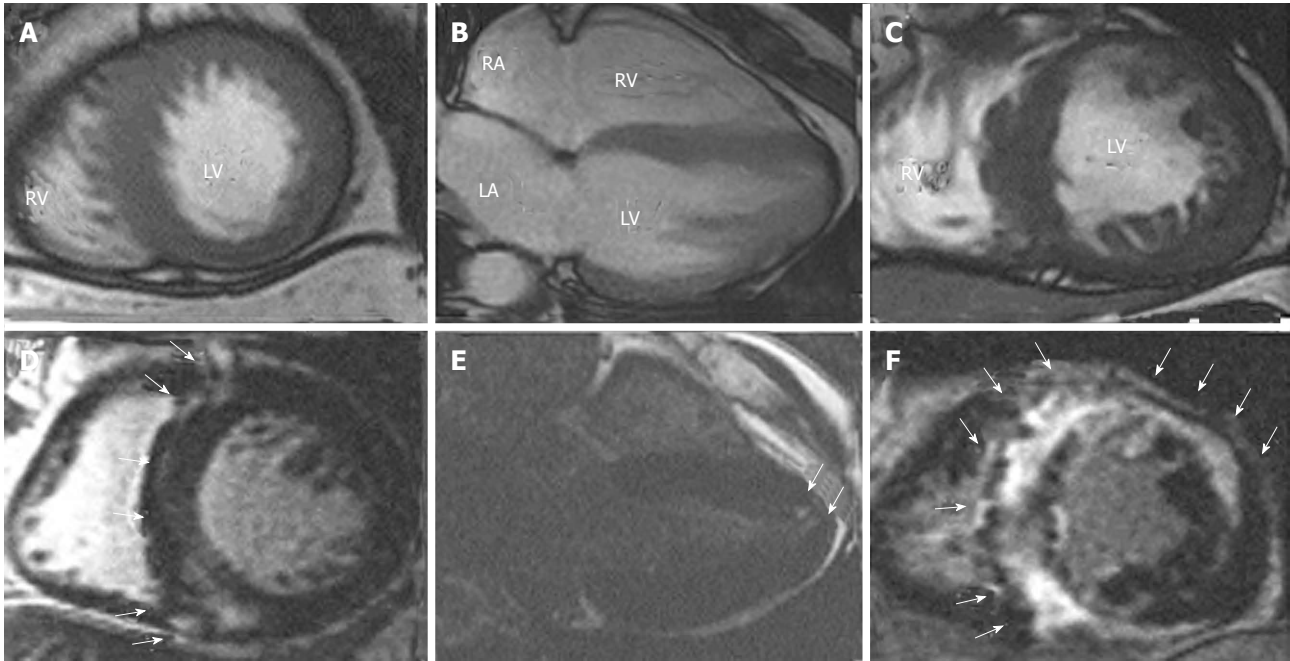


Figure 3 Representative cine-cardiac magnetic resonance (A-C) and late gadolinium enhancement-cardiac magnetic resonance (D-F) images in patients with various phenotypes of hypertrophic cardiomyopathy. A, D: ASH (short axis views); B, E: APH (horizontal views); C, F: End-stage HCM (short axis views). LGE was mainly localized in the ventricular septum and right ventricular insertion points in ASH and in the apex in APH (arrows). Note the inhomogeneous LV wall thickness and diffusely spread LGE in end-stage HCM. All the images are taken from Sato *et al.*^[11]. ASH: Asymmetrical septal hypertrophy; APH: Apical hypertrophy; LGE: Late gadolinium enhancement; LV: Left ventricular; HCM: Hypertrophic cardiomyopathy.

patients who are most likely to benefit from early aggressive therapies.

Since myocardial fibrosis may provide an arrhythmogenic underlying substrate, previous studies examined the correlation between LGE and ECG abnormalities or ventricular arrhythmias in HCM. The disturbance of conduction system, exhibited as prolonged QRS duration and/or QRS axis deviation was correlated with LGE volume and LGE distribution into inter-ventricular septum^[11,45]. Although the contribution of LGE to abnormal Q waves still remains controversial, the segmental and transmural extent rather than the mere presence of LGE may be the underlying mechanism of abnormal Q waves^[11,45,46]. The apical hypertrophy (APH) is a common type of HCM especially in Japan^[47]. The giant negative T waves are one of the characteristics of APH, and the depth of negative T waves was related to the asymmetric distal hypertrophy^[11]. We and others also reported the progression of apical myocardial damage expressed as LGE reduced the QRS voltage, the depth of negative T waves, and caused fragmentation of QRS waves^[48]. Recent studies have also shown that HCM patients with LGE are more likely to have episodes of non-sustained VTs, higher frequency of ventricular extrasystoles as well as VT inducibility in the electrophysiological study^[9,13,49].

Risk stratification in HCM is difficult because of the heterogeneity in the clinical and phenotypic expression and the low event rate^[44,49]. However, HCM is one of the most common disorders causing SCD. There are five clinically accepted high-risk factors for SCD, including a family history of sudden death, extreme LV hypertro-

phy (> 30 mm), unexplained syncope, a documentation of non-sustained VTs, and an abnormal blood pressure response during upright exercise^[50]. A recent review has shown a close relationship between LGE and cardiovascular mortality, heart failure death, and all-cause mortality in HCM^[51]. Additionally, stress perfusion CMR could be used to further stratify the risk for SCD, since inducible myocardial ischemia is another risk in HCM, which was proven by a study on single-photon emission computed tomography (SPECT)^[52].

End-stage (dilated phase) HCM

End-stage HCM, which is characterized by LV systolic dysfunction and enlargement of LV cavity, is recognized as a part of HCM disease spectrum^[53]. Since the clinical condition in end-stage HCM resembles that in DCM, the differential diagnosis of them becomes difficult, if the hypertrophy was undiagnosed or underestimated during the natural course of the disease. Patients with end-stage HCM frequently exhibit severe heart failure and lethal ventricular arrhythmias, thus resulting in higher mortality rates than the overall HCM or DCM population^[13,53]. Therefore, the early and correct recognition of those patients is necessary to start aggressive medical and device therapies.

Cine-CMR exhibits that LV wall thickness in end-stage HCM is normal or relatively larger and is inhomogeneous among LV segments compared with that in DCM (Figure 3)^[13]. LGE-CMR also shows that LGE in end-stage HCM distributes more diffusely into all the LV segments, whereas that in DCM is localized mainly in the

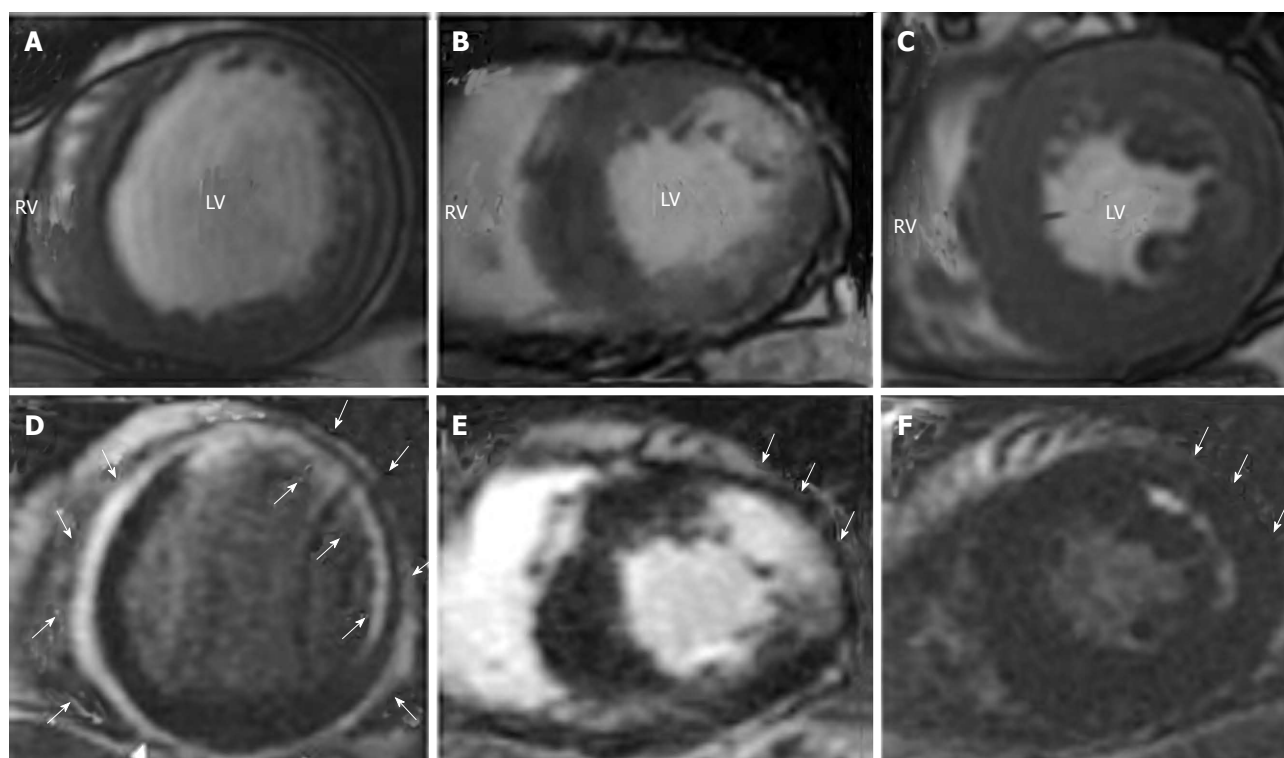


Figure 4 Representative cine-cardiac magnetic resonance (A-C) and late gadolinium enhancement-cardiac magnetic resonance (D-F) images in patients with cardiac sarcoidosis. A, D: A patient with LV dilatation, reduced LVEF (22%) and circumferential subepicardial and subendocardial LGE with spared mid-myocardium; B, E: A patient with reduced LVEF (38%) and nodular LGE in antero-lateral wall; C, F: A patient with preserved LVEF (58%) with mid-wall striated LGE in antero-lateral wall. White arrows indicate LGE areas. A part of the images is taken from Matoh *et al.*^[10] with permission. LGE: Late gadolinium enhancement; LV: Left ventricular; LVEF: Left ventricular ejection fraction

inter-ventricular septum^[6,9,11,38]. Detailed analyses of both cine-CMR and LGE-CMR can help differentiation of end-stage HCM from DCM and other secondary cardiomyopathies that exhibit LV dysfunction with hypertrophy (*e.g.*, cardiac amyloidosis and Anderson-Fabry disease).

CARDIAC SARCOIDOSIS

General

Sarcoidosis is a multi-system disorder of unknown etiology. Clinical cardiac involvement is found in only 5% to 7% of patients with sarcoidosis, whereas postmortem studies have identified myocardial lesions in 20% to 60%^[54]. Autopsy studies showed that cardiac sarcoid lesions were mainly non-transmural and located in the basal LV and subepicardial myocardium^[55-57].

The diagnosis of cardiac sarcoidosis has been made with endomyocardial biopsy, and the guideline of Japanese Ministry of health and welfare (JMH) is also based on histological diagnosis^[58]. However, biopsy results are sometimes false negative because of discrete distribution of sarcoid lesions. Hence, patients with systemic sarcoidosis and those with impaired LV function who are suspected cardiac involvement of sarcoidosis are not always positive according to the guideline. Therefore, some patients have been misdiagnosed with normal or DCM, and do not benefit from immunosuppressive therapies. Since patients with cardiac sarcoidosis have a poor prognosis, and a treatment with corticosteroid can improve long-

term prognosis, an earlier diagnosis of cardiac involvement of sarcoidosis with non-invasive imaging modalities is crucial.

CMR features

In cardiac sarcoidosis, cine-CMR can image segmental wall motion abnormalities, wall thinning, and aneurysm formation. LGE-CMR identifies LGE in the LV wall^[10,55-58]. The mechanism of LGE in cardiac sarcoidosis is considered to be heterogeneous, and may contain not only fibrotic scar but also an increased interstitial space due to the formation of non-caseating epithelioid cell granuloma^[59]. LGE in cardiac sarcoidosis may reflect irreversible myocardial damage, since we and others could not demonstrate a reduction in LGE volume during various follow-up periods^[10,58].

Previous studies compared findings between LGE-CMR and SPECT or ¹⁸F-fluorodeoxyglucose-positron emission computed tomography (FDG-PET) in the diagnosis and assessment of cardiac sarcoidosis. A previous paper noted that the transmural extent of LGE was well associated with defect scores in ²⁰¹Tl-SPECT^[56]. We found that LGE distributed mostly into the basal and mid inter-ventricular septum, but also spread into all the LV segments. Additionally, we and other investigators found that nodular, circumferential, and subepicardial and subendocardial types of LGE distribution exhibited high specificity for differential diagnosis from DCM (97%-100%, Figure 4)^[57,58]. Although the new JMH guideline includes

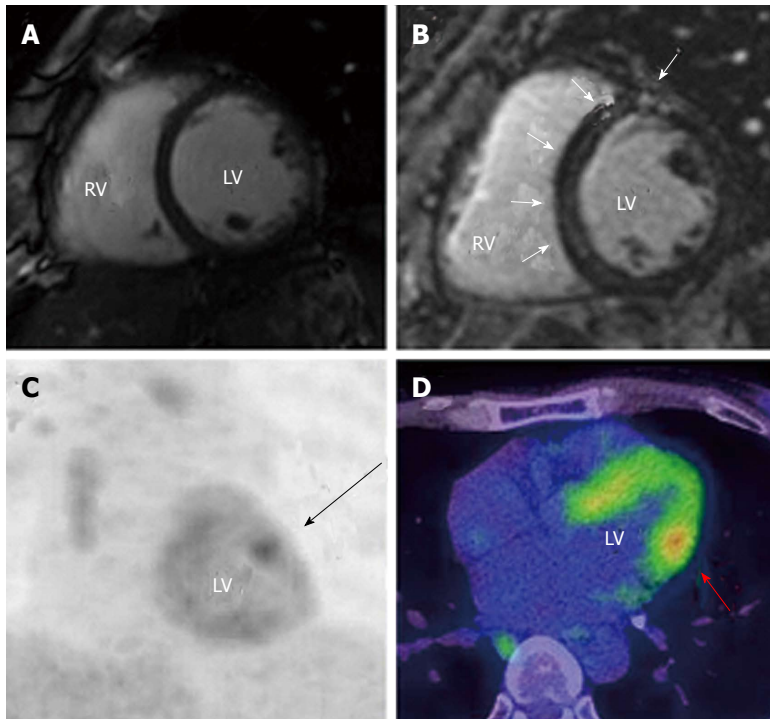


Figure 5 Representative short axis cine-cardiac magnetic resonance (A), late gadolinium enhancement-cardiac magnetic resonance (B), ^{18}F -fluorodeoxyglucose-positron emission computed tomography (C), and positron emission computed tomography (D) images in a 57-year-old male patient with systemic sarcoidosis. The diagnosis of sarcoidosis was made with liver biopsy. Cine-CMR images shows normal LV size and contraction (LVEDV: 119 mL, LVEF: 73%), but LGE-CMR reveals patchy and striated LGE in anterior wall and inter-ventricular septum (white arrows). The patient was negative for cardiac involvement of sarcoidosis according to the guideline of Japanese Ministry of Health and Welfare. However, FDG-PET and PET-CT images demonstrate hot spot in postero-lateral wall of LV, indicative of active inflammatory change (black and red arrows). FDG-PET: ^{18}F -fluorodeoxyglucose-positron emission computed tomography; LGE: Late gadolinium enhancement; LV: Left ventricular; LGE-CMR: Late gadolinium enhancement-cardiac magnetic resonance.

the presence of LGE as a minor criterion for cardiac sarcoidosis^[60], the characteristic patterns of LGE distribution may help more precise diagnosis.

T2-weighted CMR sometimes shows punctuated or patchy signals in the acute lesions of cardiac sarcoidosis with myocardial edema^[61].

Clinical implications

In sarcoidosis, patients with LGE in myocardium show heart failure symptoms, and a higher prevalence of ECG abnormalities and VTs^[58]. The correlations between LGE volume, and LV volume and function are also described. Hence, the cardiac outcome in patients with LGE is significantly lower than that without LGE^[57,58].

While LGE and defects in ^{201}Tl -SPECT represent irreversible fibro-granulomatous replacement, the hot spots in ^{67}Ga -SPECT or FDG-PET indicate active inflammatory change, which can also be used for assessing the effect of corticosteroid therapy^[62,63]. Since FDG-PET can provide better sensitivity compared with SPECT, the combination of CMR and FDG-PET may improve overall sensitivity for diagnosis and help therapeutic strategies (Figure 5)^[63,64].

STRESS (TAKOTSUBO) CARDIOMYOPATHY

General

Stress cardiomyopathy (SC), initially reported in Japan as Takotsubo cardiomyopathy, is characterized by an acute, severe but reversible LV dysfunction without significant coronary artery disease^[65,66]. The majority of patients have a clinical presentation similar to that of acute coronary syndrome (ACS)^[66]. The precise incidence of SC is unknown, but recent studies have revealed a prevalence

of approximately 2% of patients presenting ACS in the United States and Europe^[66,67]. There is a high predominance in elderly women, and several instances are possibly triggered by physical or emotional stress^[68,69]. Despite severe presentation in acute phase, complications are rare and the prognosis of patients with SC is generally considered favorable^[67,70].

Although the mechanism of SC has not yet been fully clarified, considerable evidence suggests that enhanced sympathetic activity might play a pathogenic role in the transient myocardial dysfunction observed in SC^[71]. At the tissue level, myocardial edema as a sign of acute but reversible injury and diffuse inflammation in the absence of significant necrosis/fibrosis are characteristics of SC. However, other histological analyses of the heart in SC showed sparse foci of myocardial necrosis with contraction bands in the akinetic area^[71,72].

CMR features

CMR at acute phase (approximately 5 d after onset) is mostly suited for the evaluation of patients with SC. Since CMR imaging can provide markers for reversible and irreversible injury, it may be particularly important to diagnose SC from ACS and myocarditis^[66,70,73].

A previous study suggested diagnostic criteria with CMR: (1) severe LV dysfunction in a non-coronary regional distribution pattern; (2) myocardial edema collocated with the regional wall motion abnormality; (3) absence of high-signal areas in LGE images; and (4) increased early myocardial gadolinium uptake^[66]. The LV dysfunction in cine-CMR is typically apical ballooning shape with akinesis of apical and mid-ventricular LV segments (so-called Takotsubo-like). However, fewer patients presented a mid-ventricular variant with apical sparing or with isolated basal ballooning^[66,69]. Mean LVEF was 39%

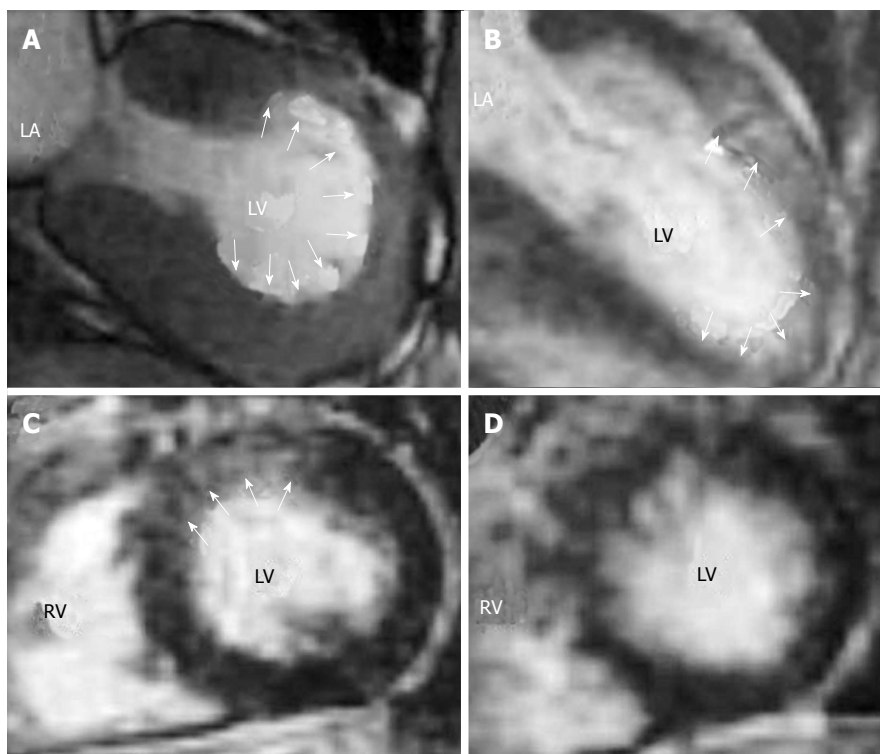


Figure 6 Representative cine-cardiac magnetic resonance (A) and late gadolinium enhancement-cardiac magnetic resonance (B-D) images in a case of stress (Takotsubo) cardiomyopathy. The images show vertical long axis (A, B) and mid-ventricular short axis (C, D) views. The cine-CMR image during systole (A) shows mid-anterior dyskinesia (white arrows). LGE-CMR images on the sub-acute phase (B, C) show that the area of LGE was well matched with the area of wall motion abnormality (white arrows). On the follow-up phase, LV systolic function recovered, and the LGE-CMR image (D) could not detect significant LGE in the LGE area observed on the sub-acute phase. All the images are taken from Naruse *et al*^[69] with permission. LGE: Late gadolinium enhancement; LV: Left ventricular; LGE-CMR: Late gadolinium enhancement-cardiac magnetic resonance.

in the acute setting and 65% in the recovery phase. Cine-CMR also clarified right ventricular (RV) dysfunction in 38.5% of patients, and apical thrombus in 5.1%^[73]. T2-weighted images can also show myocardial edema co-located with the regional wall motion abnormality^[66]. The absence of LGE has been described in many case studies and is a common diagnostic criterion^[66,70]. However, a recent meta-analysis has demonstrated LGE in a certain part of cases with SC^[73]. A previous study showed evidence for the immune-histological basis of the LGE phenomenon in patients with SC^[74].

We found LGE in 8 of 20 patients with SC^[69]. The signal intensity was lower than that usually documented in cases of myocardial infarction or myocarditis (Figure 6). Another study also showed that focal and patchy LGE was detected in a certain part of patients when using a threshold of 3 standard deviation (SD) instead of 5 SD above the mean of remote myocardium to define significant enhancement^[66]. Possible speculations are that severe stress-induced stunning of the apical segments leads to a patchy pattern of myocardial contraction-band necrosis possibly accompanied by a certain amount of transient focal/patchy edema or deposition of extracellular matrix resulting in LGE with low signal intensity. We also detected LGE at the recovery phase in fewer patients.

Clinical implications

Although the LV dysfunction in SC is mostly reversible,

an involvement of RV is associated with longer hospitalization, heart failure, and older age. Cine-CMR can clarify the exact incidence of bi-ventricular ballooning^[66,75]. We also showed that patients with LGE experienced cardiogenic shock more frequently and had a longer duration to ECG normalization and recovery of wall motion than did those without LGE^[69]. Contrary, another study exhibited that the presence of less rigorously defined LGE during the acute phase had no persisting effect on global LV function, and there was no evidence of LGE at CMR follow-up^[66]. Thus, the clinical implications of such type of LGE remain still elusive. In both studies, however, the absence of significant LGE was consistent with the complete normalization of LV function in patients with SC.

OTHER CARDIOMYOPATHIES

Arrhythmogenic right ventricular cardiomyopathy/dysplasia

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a disease of heart muscle characterized by structural and functional abnormalities of RV wall due to replacement of the myocardium by fatty and fibrous tissue. This disorder is relatively uncommon but life-threatening cardiomyopathy with progressive RV failure, ventricular arrhythmias and SCD. Although RV is the predominantly diseased chamber, LV can also be the affected chamber in some cases^[76].

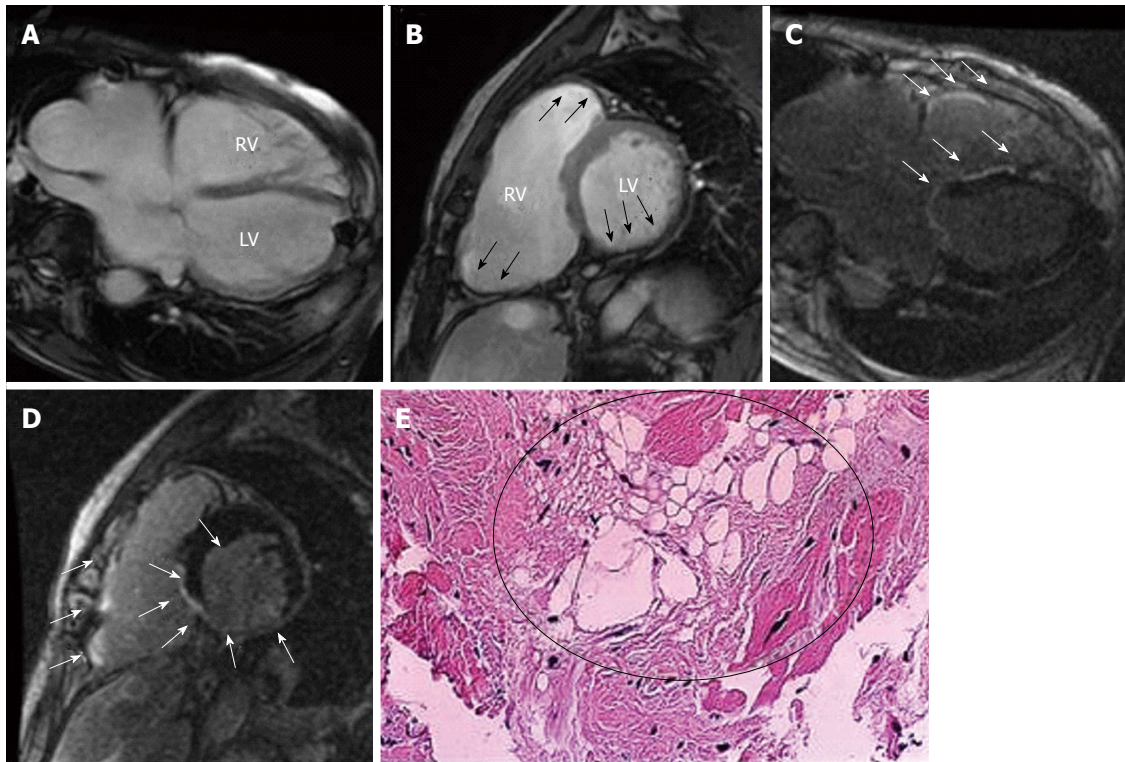


Figure 7 Representative cine- cardiac magnetic resonance (A, B) and late gadolinium enhancement-cardiac magnetic resonance (C, D) images in a 55- year-old male patient with arrhythmogenic right ventricular cardiomyopathy/dysplasia. The images show horizontal axis (4-chambers) (A, C) and mid-ventricular short axis (B, D) views. Cine-CMR images reveal dilatation of both RV and LV chamber. Focal dilatation of RV and wall thinning in inferior LV wall are also apparent (black arrows). LGE-CMR images show diffuse LGE in RV wall and in inferior LV wall (white arrows). A sub-endocardial biopsy demonstrates fatty infiltration in RV myocardium (Circle, H-E stain, 100×). LGE: Late gadolinium enhancement; LV: Left ventricular; LGE-CMR: Late gadolinium enhancement-cardiac magnetic resonance; RV: Right ventricular.

The diagnosis of ARVC/D is challenging due to heterogeneous clinical presentation and non-specific ECG findings^[77,78]. The diagnosis is currently made on the presence of major and minor Task Force criteria that include structural, functional, histological, electrocardiographic, arrhythmic, and genetic factors^[79]. Endomyocardial biopsy is considerably unreliable for the diagnosis of ARVC/D, because the patchy distribution of the fibro-fatty change may cause sampling error.

CMR can visualize RV wall better than echocardiography. Functional abnormalities in cine-CMR include regional wall motion defects, focal aneurysms, global RV dilation and dysfunction^[1,21]. In addition, the diagnosis could be supported by the presence of fatty infiltration of RV free wall that can be suppressed in fat suppression sequences^[2,21]. LGE imaging has been shown to provide additional evidence of fibrosis which often co-exists in the fat-infiltrated RV myocardium (Figure 7).

Despite the limitations of thin RV wall and small volume of affected myocardium, CMR frequently identifies individuals with early disease, in whom Task Force criteria are relatively insensitive^[21]. The presence of LGE can also predict inducible VTs on electrophysiological studies^[80].

Cardiac amyloidosis

Cardiac involvement has been described in most forms

of amyloidosis, but is most common and clinically significant in type AL amyloidosis (primary amyloidosis)^[81]. Cardiac amyloidosis is a common cause of restrictive cardiomyopathy, and reduced ventricular wall compliance leads to impairment of diastolic filling and diastolic heart failure even when systolic function was preserved. At a histological level, amyloidosis is evident by extra-cellular deposition of insoluble fibrillar proteinaceous material (amyloid fibrils) in various cardiac tissues including valve leaflets and coronary vessels.

On cine-CMR, diffuse myocardial hypertrophy including both ventricles and atria is seen with thickened valve leaflets and pericardial effusion. The accumulation of amyloid fibrils in the myocardial interstitium also results in unique LGE appearances. In the early disease stage, a characteristic subendocardial enhancement of LV and RV, sparing the mid-wall of the inter-ventricular septum has been reported. However, as the accumulation of amyloid fibrils expands interstitial space, the volume of distribution of gadolinium increases. Therefore, there is usually a homogeneous pattern of enhancement, such that the signal from the myocardium cannot be adequately suppressed and differentiated from the adjacent blood pool. Actually, previous studies showed an atypically dark appearance of the blood pool, which reflects the similar myocardial and blood T1 values attributable to high myocardial uptake and fast blood pool washout (Figure 8)^[1,2,82].

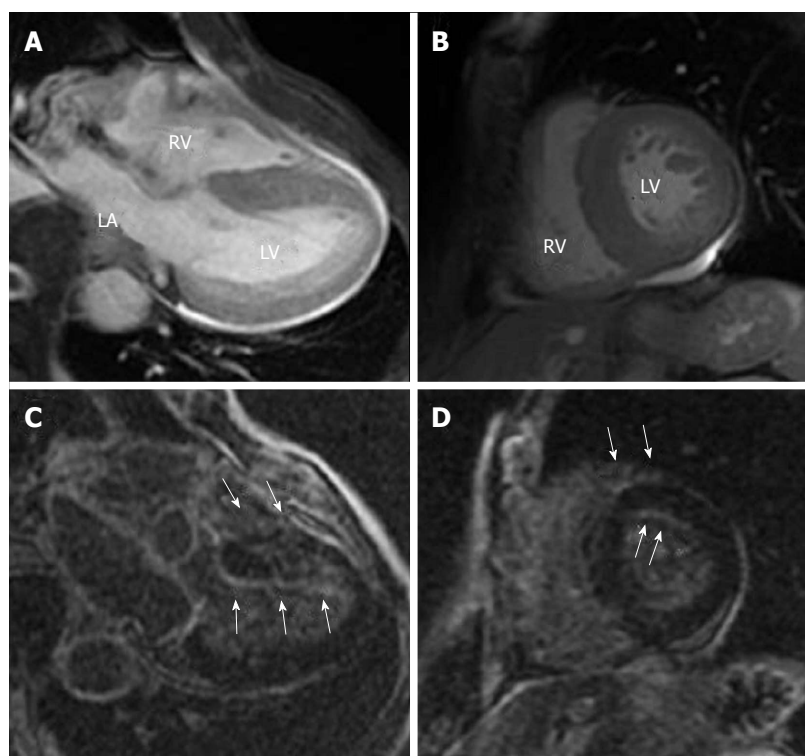


Figure 8 Representative cine-cardiac magnetic resonance (A, B) and Late gadolinium enhancement-cardiac magnetic resonance (C, D) images in a 76-year-old male patient with AL amyloidosis (IgA type multiple myeloma, Bence-Jones protein positive). The images show horizontal axis (4-chambers) (A, C) and mid-ventricular short axis (B, D) views. Cine-CMR images reveal diffuse hypertrophy in LV and RV wall. LGE-CMR images show a characteristic subendocardial enhancement of the LV and RV with an atypically dark appearance of the blood pool (white arrows). LGE: Late gadolinium enhancement; LV: Left ventricular; LGE-CMR: Late gadolinium enhancement-cardiac magnetic resonance; RV: Right ventricular.

A recent study has also demonstrated a potential of remarkable prolongation of non-contrast (native) T1 in AL amyloidosis^[83].

A positive CMR finding, that is biventricular hypertrophy, characteristic LGE distribution, and pericardial effusion, is associated with poor outcomes (heart failure and death) in patients with AL amyloidosis^[82,84].

Myocarditis

Myocarditis is most commonly caused by a viral infection resulting in myocardial inflammation and immune-mediated damage in cardiomyocytes. Acute myocarditis causes chest pain, ST-T changes and elevated cardiac enzymes, which are sometimes difficult to be differentiated from ACS, and is occasionally complicated by fulminant heart failure and SCD^[85]. Chronic myocarditis is one of the common causes of NICM, and sometimes misdiagnosed as DCM^[1].

The most characteristic features in CMR are the presence of myocardial edema, diffuse wall motion abnormalities, subepicardial patchy myocardial LGE, and the concomitant involvement of the pericardium^[86,87]. Edema imaging using T2 black blood sequences plays an important role in the evaluation of patients with suspected myocarditis^[20,61]. Edema should be verified by a quantitative signal intensity analysis, best by calculating the ratio between myocardium and skeletal muscle. Early gadolinium enhancement and prolonged native T1 are also indicative of myocardial edema^[20,88]. On LGE-CMR, the subepicardial layer especially in postero-lateral wall has LGE, and in severe cases, LGE may be more diffuse and circumferential^[89].

Anderson-fabry disease

Anderson-fabry disease (AFD) is an X-linked lysosomal

storage disorder caused by the partial or complete deficiency of α -galactosidase A. The enzymatic deficit results in progressive intracellular accumulation of excess cellular glycosphingolipid substrate in multiple organs^[90]. Cardiac involvement in AFD is frequent, and the myocardial accumulation of glycosphingolipids acts as a trigger leading to myocardial cell hypertrophy and interstitial fibrosis. Hence, most patients present LV hypertrophy, and often exhibit conduction defects, supra-ventricular and ventricular arrhythmias, and heart failure symptoms associated with progressive LV dysfunction^[91,92]. The presence and extent of cardiac damage increase progressively with age. Enzyme replacement therapy with recombinant α -galactosidase A clears microvascular deposits of glycosphingolipids, and several recent studies have shown a reduction in LV hypertrophy and improvement in systolic function after treatment^[93,94].

Therefore, differentiating AFD from other causes of LV hypertrophy is critical but is usually difficult on common imaging modalities including echocardiography. A binary endocardial appearance, initially expected as a highly sensitive and specific finding in AFD, was later ascertained to be insufficient for a screening tool^[95,96].

Instead, CMR has become a promising tool to diagnose cardiac involvement of AFD. Cine-CMR can exhibit a symmetrical and non-obstructive LV hypertrophy, and LGE-CMR can demonstrate a particular LGE distribution to the infero-lateral wall of mid to basal LV and to mid-myocardial layer (Figure 9)^[92,97]. Furthermore, a recent non-contrast T1 mapping technique has potential to detect early cardiac involvement of AFD by showing T1 shortening^[98]. Thus, AFD should always be considered if unexplained LV hypertrophy is seen, particularly in a young patient with family history.

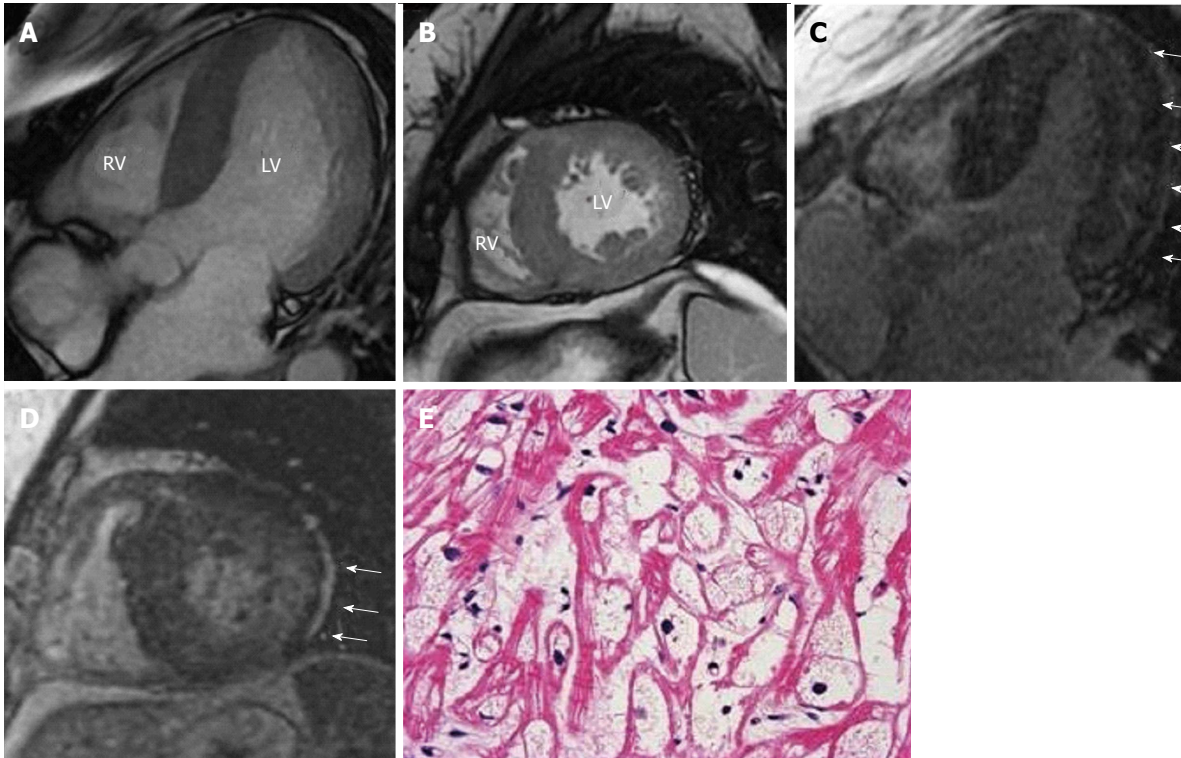


Figure 9 Representative cine- cardiac magnetic resonance (A, B) and late gadolinium enhancement-cardiac magnetic resonance (C, D) images in a 46-year-old female patient with Anderson-fabry disease. The images show horizontal axis (4-chambers) (A, C) and mid-ventricular short axis (B, D) views. Cine-CMR images reveal diffuse hypertrophy of LV wall. LGE-CMR images show a particular LGE distribution pattern to the infero-lateral mid to basal segments and to mid-myocardial layer (white arrows). E: A sub-endocardial biopsy from RV wall demonstrates interstitial fibrosis and cardiomyocyte hypertrophy with cytoplasmic vacuolization (H-E stain, 40 \times). LGE: Late gadolinium enhancement; LV: Left ventricular; LGE-CMR: Late gadolinium enhancement-cardiac magnetic resonance; RV: Right ventricular.

Endomyocardial fibrosis

Endomyocardial fibrosis (EMF) is the most frequent restrictive cardiomyopathy especially affecting poor children and young adults in the tropical zone. The characteristic features are fibrotic tissue deposition in the endocardium of the inflow tract and apex of one or both ventricles. The pathogenesis of EMF is poorly understood, but early hyper eosinophilia may play a role^[99].

Cine-CMR can clearly demonstrate distorted ventricles with normal or reduced volume and enlarged atria. LGE-CMR can also show areas of LGE in the endocardium where the histopathological examination revealed extensive fibrous thickening, proliferation of small vessels and scarce inflammatory infiltrate. The LGE pattern may have a “V sign” at the ventricular apex, characterized by a 3-layer appearance of myocardium, thickened fibrotic endocardium, and overlying thrombus^[100]. The relationships between increased LGE burden and worse NYHA functional classes, and increased probability of surgery and mortality rate are reported^[100].

Since the reports of EMF have been increasing in areas where the disease had not been previously recognized, the role of CMR may increase for the early diagnosis of EMF^[101].

Systemic sclerosis

Systemic sclerosis (SSc) is characterized by vascular changes and fibrosis of the skin and internal organs.

Among many autoimmune disorders, SSc has been considered to have a high prevalence of cardiac involvement. The prevalence is clinically 1.4% to 5.4% for systolic or 18% to 30% for diastolic dysfunction^[102,103]. While in autopsy, myocardial fibrosis was identified in 50% to 80%^[104]. Cardiac involvement in SSc is assumed to be derived from impairment of the microcirculation and primary myocardial fibrosis, and from ischemic damage due to coronary atherosclerosis^[105,106]. Patients with cardiac involvement have a poor prognosis because of congestive heart failure and fatal arrhythmias associated with conduction disturbance^[107]. Unfortunately, most patients with cardiac involvement are asymptomatic and difficult to be detected in subclinical stage.

Recently, the values of CMR are suggested for the early detection of cardiac involvement in SSc. Actually, previous reports revealed LGE in 21% to 66% of patients with SSc^[108-110]. LGE distributed mainly into the basal to mid inter-ventricular septum and RV insertion points, and spread into all the myocardial layers, reflecting various mechanisms for myocardial fibrosis.

We showed the correlations between LGE and enlargement of LV/RV volume, impaired LV/RV function and pulmonary arterial hypertension. The ability of LGE-CMR to detect cardiac fibrosis in the subclinical stage may help identification of high risk patients and early initiation of therapeutic interventions, although the relevance in long term prognosis remains to be elucidated.

Table 1 Distribution and patterns of late gadolinium enhancement and other cardiac magnetic resonance findings in various types of cardiomyopathies

Cardiomyopathies		LGE distribution		LGE patterns	Other CMR features
		Intra-cardiac	Intra-myocardium		
Ischemic		LV regions corresponding to coronary distribution	Subendocardium to transmural	Striated, transmural	LV wall motion abnormality, wall thinning, aneurysm
Non-ischemic	Dilated cardiomyopathy	Inter-ventricular septum	Mid-wall	Striated	Diffuse LV wall thinning, shortened post-contrast T1
	Hypertrophic cardiomyopathy	Regions with hypertrophy	Any	Patchy, striated	Asymmetrical or symmetrical LV hypertrophy
	End-stage HCM	Diffuse	Any	Patchy, striated, transmural	LV dilatation with Inhomogenous LV wall thickening
	Cardiac sarcoidosis	Any	Any	Patchy, striated, transmural	LV aneurysm, myocardial edema in T2 black blood sequences
	Stress cardiomyopathy	Regions with ballooning	Any	Patchy, transient	Myocardial ballooning, RV motion abnormality
	Arrhythmogenic right ventricular cardio-myopathy/dysplasia	RV (sometimes with LV)	Any	Striated	Focal or global RV dilatation, fatty infiltration in fat suppression sequence
	Cardiac amyloidosis	Any	Subendocardial, transmural	Diffuse	Diffuse hypertrophy, thickened valve leaflets, prolonged native T1
	Myocarditis	Any	Subepicardial	Diffuse	Myocardial edema in T2 black blood sequences, early GE, prolonged native T1
	Anderson-Fabry disease	Postero-lateral LV	Mid-wall	Striated	Concentric/eccentric but non-obstructive LV hypertrophy, shortened native T1
	Endomyocardial fibrosis	Inflow tract to apex	Subendocardial	Diffuse	Distorted ventricles with normal or reduced volume and enlarged atria
	Cardiac involvement in SSc	Any	Any	Any	
	LV non-compaction	NA	NA	NA	High non-compacted/compacted myocardial ratio
	Iron-overload cardio-myopathy (cardiac hemochromatosis)	NA	NA	NA	Shortened T2-star

LV: Left ventricular; LGE: Late gadolinium enhancement; CMR: Cardiac magnetic resonance; RV: Right ventricular; NA: No available information; ICM: Ischemic; NICM: Non-ischemic; DCM: Dilated cardiomyopathy; HCM: Hypertrophic cardiomyopathy.

Table 1 summarizes the typical distribution and patterns of LGE, and other characteristic CMR features in ICM and NICM. In addition to above mentioned NICM, cine-CMR can show clearer images in terms of the presence of apical trabeculations, deep inter-trabecular recesses and high non-compacted/compacted myocardial ratio in patients with LV non-compaction^[11]. In addition, a T2* technique allows to estimate iron deposition in myocardium, and to correlate it with cardiac function and the effect of chelation in iron overload cardiomyopathy (cardiac hemochromatosis)^[19].

LIMITATION OF CMR

Despite the benefits with much evidence, CMR is not necessarily available in all institutes and patients, and has a problem of cost. Claustrophobia is a frequent reason to cancel MRI. Patients with decompensated heart failure cannot be tolerant to long data acquisition time of MRI. MRI is still contraindicated in patients who have had device implantation (*e.g.*, permanent pacemaker, implantable cardioverter defibrillator or cardiac resynchronization therapy with and without defibrillation). Furthermore, gadolinium contrast agents cannot be administered to

patients with chronic renal failure because of the risk of nephrogenic systemic fibrosis. The determination of threshold and quantification of LGE are also limitations in NICM.

CONCLUSION

Currently, CMR has become one of the most important methods to diagnose and follow-up patients with ICM and NICM. This review showed that the analysis of LGE distribution in myocardium is particularly valuable for differential diagnosis and risk stratification. However, the differential diagnosis of cardiomyopathies should be made generally on the basis of combination of various CMR sequences and with other imaging modalities and endomyocardial biopsy.

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WJC 6th Anniversary Special Issues (3): Cardiomyopathy

Takotsubo cardiomyopathy: Pathophysiology, diagnosis and treatment

Kazuo Komamura, Miho Fukui, Toshihiro Iwasaku, Shinichi Hirotani, Tohru Masuyama

Kazuo Komamura, Miho Fukui, Toshihiro Iwasaku, Shinichi Hirotani, Tohru Masuyama, Cardiovascular Division, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya 663-8501, Japan

Author contributions: Komamura K designed and wrote the paper; Fukui M and Iwasaku T acquired clinical data; Hirotani S criticized intellectual content; Masuyama T finally approved the paper.

Correspondence to: Kazuo Komamura, MD, PhD, Cardiovascular Division, Department of Internal Medicine, Hyogo College of Medicine, 1-1, Mukogawa-cho, Nishinomiya 663-8501, Japan. komamura@hyo-med.ac.jp

Telephone: +81-798-456553 Fax: +81-798-456551

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Core tip: Takotsubo cardiomyopathy (TCM) is an important disease entity that differs from acute myocardial infarction. It occurs more often in postmenopausal elderly women, is characterized by a transient hypokinesis of the left ventricular (LV) apex, and is associated with emotional or physical stress. Wall motion abnormality of the LV apex is generally transient and resolves within a few days to several weeks. The prognosis of TCM is generally good. It has been suggested that coronary spasm, coronary microvascular dysfunction, catecholamine toxicity and myocarditis might contribute to the pathogenesis of TCM. However, its pathophysiology is not clearly understood.

Abstract

In 1990, takotsubo cardiomyopathy (TCM) was first discovered and reported by a Japanese cardiovascular specialist. Since then, this heart disease has gained worldwide acceptance as an independent disease entity. TCM is an important entity that differs from acute myocardial infarction. It occurs more often in postmenopausal elderly women, is characterized by a transient hypokinesis of the left ventricular (LV) apex, and is associated with emotional or physical stress. Wall motion abnormality of the LV apex is generally transient and resolves within a few days to several weeks. Its prognosis is generally good. However, there are some reports of serious TCM complications, including hypotension, heart failure, ventricular rupture, thrombosis involving the LV apex, and torsade de pointes. It has been suggested that coronary spasm, coronary microvascular dysfunction, catecholamine toxicity and myocarditis might contribute to the pathogenesis of TCM. However, its pathophysiology is not clearly understood.

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INTRODUCTION

Takotsubo cardiomyopathy (TCM) is a transient wall motion abnormality of the left ventricular (LV) apex accompanied with emotional or physical stress that usually resolves completely. Takotsubo is a Japanese word meaning a pot with a narrow neck and a round bottom used to catch octopuses. Left ventriculography during systole of patients with TCM demonstrates such a shape. Although TCM is a novel concept, the number of cases reported is increasing rapidly. Other words have been used to refer this cardiomyopathy, including stress-related cardiomyopathy^[1], transient LV apical ballooning syndrome^[2,3], broken heart (heartbreak) syndrome, and ampulla cardiomyopathy^[4]. In

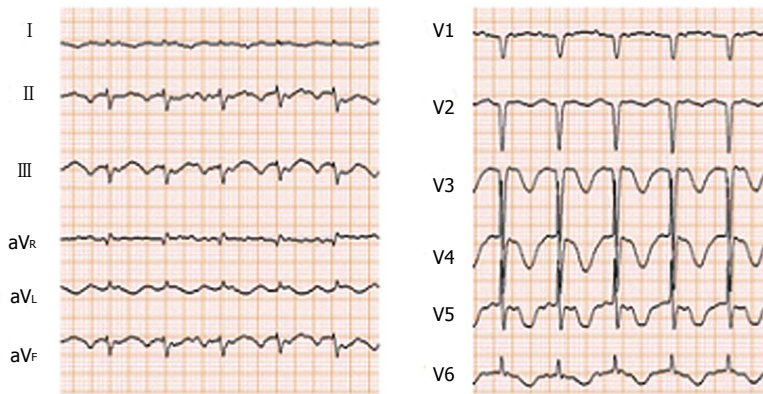


Figure 1 Inverted T waves are found in the limb and precordial leads, which is a common characteristic of takotsubo cardiomyopathy with apex balloon-like dilation.

2006, the American Heart Association incorporated this disease under the class of acquired cardiomyopathies^[5]. This article aimed to review this newly recognized cardiomyopathy, paying particular attention to clinical characteristics, pathophysiology, diagnosis, and treatment.

EPIDEMIOLOGY

TCM symptoms were considered extremely rare until the past 20 years. The increasing number of medical reports on these symptoms has highlighted the higher incidence of TCM than that previously reported. Currently, 1000 or more studies reporting cases of TCM have been published. According to a retrospective review, patients with TCM accounted for approximately 2% of all the patients with suspected acute coronary syndrome^[6,7]. Further, 90% of these patients were postmenopausal women^[8,9]. A few reports indicated that the average age of TCM patients was 68 years, although children or young adults may also be affected^[10,11]. Another report indicated that most men with TCM were inpatients, which suggests that physical stresses might play a role for the progress of the disease^[12]. In a recent study, demographic and clinical course data in patients with TCM were compared between the United States and Japan. Few Japanese patients with TCM had a history of overt coronary disease (CAD) and family history of early-onset CAD. However, there was no significant difference in long-term prognosis and the recurrence rate between the United States and Japanese patients with TCM^[13].

DIAGNOSIS

The diagnosis of TCM remains controversial. The diagnostic criteria most widely accepted were published by the Mayo Clinic^[14] in 2004. In 2008, a new criterion was added to them: a normal epicardial coronary artery (Table 1)^[15]. Kawai *et al.*^[16] classified this disease as a syndrome of unknown etiology that was characterized by acute balloon-like dilation in the LV apex (Table 2). As shown by these two diagnostic criteria, the patients with TCM have nonspecific or normal findings on physical examination; however, the clinical course resembles that of acute coronary syndrome or acute decompensated heart fail-

ure^[14-16]. The most common presenting symptoms listed in the diagnostic criteria are chest pain and dyspnea. In rare cases, patients developed palpitations, nausea, vomiting, syncope, or cardiogenic shock^[14-16].

The following six symptoms are especially indicative of TCM: (1) acute onset and stressful inducement: One of the unique features of TCM is its relation with stressful emotional or physical events. This characteristic was described in nearly two-thirds of the patients who developed TCM^[17]. Unlike acute coronary syndrome, with an onset peak early in the morning, TCM presents in the afternoon in most cases when stressful inducible events are likely to occur; (2) electrocardiographic characteristics: Although the initial electrocardiogram (ECG) of patients with TCM is nonspecific, an ST segment elevation can be found mainly in the precordial leads in 50% of patients at onset^[18,19]. In addition, reciprocal ST-segment depression in the inferior wall leads is unlikely^[20]. In comparison with patients with base deformity, inverted T waves are more frequently observed in patients with apex balloon-like dilation^[21] and they resolve spontaneously within a few weeks to several months (Figure 1). Furthermore, patients with TCM usually present abnormal Q waves in precordial leads. These Q waves are transient in most patients and generally resolve within a few days to several weeks^[22]; (3) cardiac enzymes: In most patients with TCM, there is slight elevation in the cardiac enzyme level on admission^[6,20]. The enzyme levels decrease rapidly and do not seem to have prognostic significance^[22]; (4) absence of coronary lesion: It is characteristic that no specific coronary lesions are detected in TCM^[23,24]. Generally, patients with TCM have chest pain, changes in ECG, elevation of cardiac enzyme levels, and wall motion abnormalities. Therefore, coronary angiography has to be conducted to rule out acute coronary syndrome; (5) balloon-like dilation of the ventricle: In contrast with acute myocardial infarction, LV wall motion abnormalities are found beyond a single coronary artery perfusion area in patients with TCM. Most patients with TCM show loss of motion or hypokinesia at the apex and an apical balloon-like dilation pattern associated with preservation of the base (Figure 2). However, cases of a TCM subtype without abnormalities of the apex were reported recently^[25,26]. TCM is essentially characterized by LV failure,

Table 1 Diagnostic criteria of the Mayo Clinic

Suspicion of AMI based on precordial pain and ST elevation observed on the acute-phase ECG
Transient hypokinesia or akinesia of the middle and apical regions of the LV and functional hyperkinesia of the basal region, observed on ventriculography or echocardiography
Normal coronary arteries confirmed by arteriography (luminal narrowing of less than 50% in all the coronary arteries) in the first 24 h after the onset of symptoms
Absence of recent significant head injury, intracranial hemorrhage, suspicion of pheochromocytoma, myocarditis, or hypertrophic cardiomyopathy

AMI: Acute myocardial infarction; ECG: Electrocardiogram; LV: Left ventricular.

although, approximately, one-third of patients also have abnormalities in the right ventricle^[27]. Cardiac magnetic resonance imaging (MRI) is a suitable method to establish the diagnosis of TCM because this modality allows the accurate identification of reversible myocardium damage by visualization of wall motion abnormalities in each area, quantification of ventricular function, and assessment of inflammation and fibrosis. This modality brings new insight into the pathophysiology of TCM. It could enable early treatment of acute symptoms, raise awareness, and improve clinical outcomes. Cardiac MRI is appropriate to evaluate wall motion abnormalities and LV ejection fraction, and to confirm the absence of delayed gadolinium enhancement in patients with TCM. This allows differentiation of TCM from myocardial infarction and myocarditis, both pathologies associated with delayed gadolinium enhancement^[17]. Although coronary computed tomography angiography is not applicable to the first diagnosis of patients with TCM, there are many reports on its use for clinical course evaluation after TCM onset; (6) recovery of cardiac function: One of the characteristics of TCM is that thorough recovery of cardiac function is achieved. In contrast to other serious wall motion abnormalities at onset, recovery of ventricular function is proven in follow-up evaluations. Most patients with TCM show significant improvement of systolic function within a week and achieve complete recovery by the end of third or fourth week after onset. Generally, another diagnosis should be considered in patients with suspected TCM whose systolic function is not normalized within 12 wk after onset.

The differential diagnosis of TCM includes the following: esophageal spasm, gastroesophageal reflux disease, myocardial infarction, myocardial ischemia, unstable angina, acute coronary syndrome, angina, aortic dissection, myocarditis, acute pericarditis, pneumothorax, cardiogenic pulmonary edema, pulmonary embolism, Boerhaave syndrome (spontaneous esophageal rupture), cardiac tamponade, cardiogenic shock, cocaine-induced cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, and coronary artery spasm.

PATHOPHYSIOLOGY

The exact pathogenesis of TCM is unknown, but various

Table 2 Diagnostic criteria of Kawai *et al.*^[16]

Exclusion criteria
Significant organic stenosis or spasm of a coronary artery. In particular, AMI due to a lesion of the anterior descending artery of the left coronary artery, which irrigates a large territory including the apex of the LV (urgent coronary angiography is desirable in order to view the image in the acute phase; during the chronic phase, coronary angiography is necessary to confirm the presence or absence of significant stenotic lesions or abnormal lesions that could explain the ventricular contraction)
Cerebrovascular disturbances
Pheochromocytoma
Viral or idiopathic myocarditis
(Note: Coronary angiography is required for the exclusion of coronary artery lesions. Takotsubo-like myocardial dysfunction can occur in conditions such as cerebrovascular disorders or pheochromocytoma)
Diagnostic references
Symptoms: Precordial pain and dyspnea similar to the findings in the acute coronary syndrome. TCM can also occur without symptoms
Triggers: Emotional or physical stress, although it can also occur without any obvious trigger
Age and gender: There is a recognized tendency to a higher frequency in elderly individuals, principally women
Ventricular morphology: Apical ballooning with rapid recovery on ventriculography and echocardiography
ECG: ST elevation may be observed immediately after the event. T waves progressively become negative in various leads and the QT interval progressively lengthens. These changes gradually improve, but the T waves may remain negative for months. Pathological Q waves and alterations of the QRS voltage may be observed in the acute phase
Cardiac biomarkers: There is only a slight rise in the cardiac enzymes and troponin
Nuclear medicine scan of the heart: Abnormalities may be detected on myocardial gamma scan in some cases
Prognosis: Recovery is rapid in most cases, but some patients develop acute pulmonary edema and other sequel, even death

AMI: Acute myocardial infarction; ECG: Electrocardiogram; LV: Left ventricular; TCM: Takotsubo cardiomyopathy.

hypotheses have been suggested and discussed, including coronary microvascular dysfunction, coronary artery spasm, catecholamine-induced myocardial stunning, reperfusion injury following acute coronary syndrome, myocardial microinfarction and abnormalities in cardiac fatty acid metabolism. Currently, catecholamine-induced cardiotoxicity and microvasculature dysfunction are the most supported theories.

Catecholamine theory (Figure 3)

Wittstein *et al.*^[22] found that the serum catecholamine concentration was two to three times greater in patients with TCM than that in patients with myocardial infarction, and described that serious emotional stress is a precipitating factor. It has been reported that exogenously administered catecholamines and pheochromocytoma cause typical characteristics of TCM, which supports this theory further^[28,29].

Lyon *et al.*^[30] advocated a theory called “stimulus trafficking” that could explain the decline of myocyte contractile function in patients with TCM. Supraphysiological levels of catecholamines induce β_2 -coupling from Gs to Gi. Therefore, the decline of myocyte contractile func-

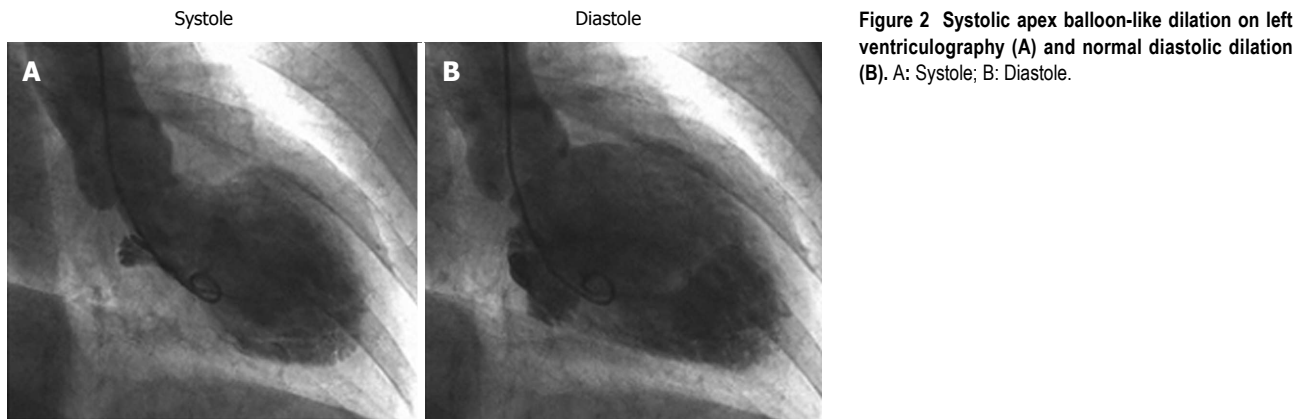


Figure 2 Systolic apex balloon-like dilation on left ventriculography (A) and normal diastolic dilation (B). A: Systole; B: Diastole.

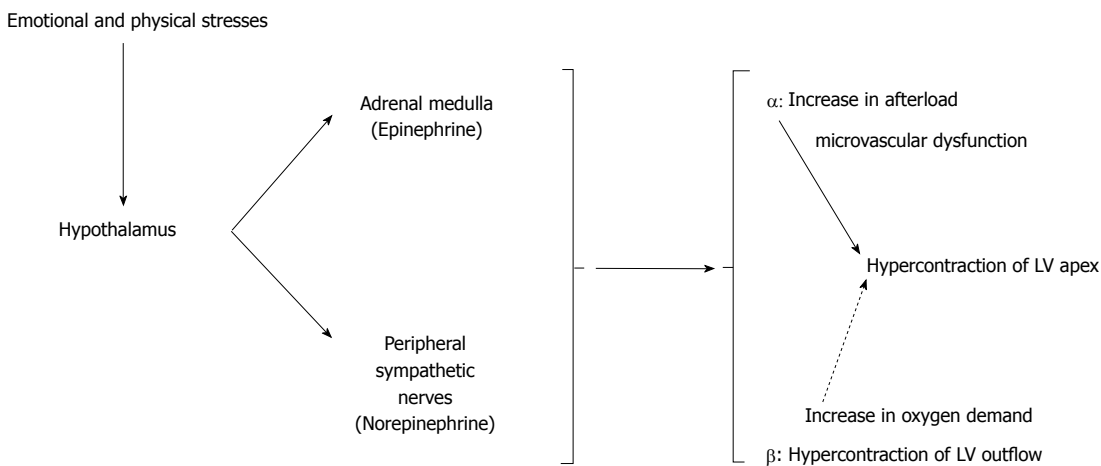


Figure 3 The catecholamine theory of takotsubo cardiomyopathy. LV: Left ventricular.

tion is evidenced by hypokinesia in ECG. Involvement of the apex can be attributed to higher adrenoceptor density in the apex than in the base^[31]. The rationale of stimulus trafficking is that a switch to Gi occurs to protect the myocytes from the strong stimulation of Gs, which causes apoptosis. Slow increases in serum troponin level explain early minimal necrosis of the myocardial tissue. Nef *et al*^[32] showed increased activity of the phosphatidylinositol 3-kinase-protein kinase B (PI3K/AKT) signaling pathway, which has important anti-apoptosis functions and plays a role in the rapid recovery of myocytes. Thus, the transient LV dysfunction can be attributed to the PI3K/AKT pathway and inversely switching from Gi to Gs, associated with the homogeneous, prompt and clinically thorough recovery of systolic function observed in TCM.

Patients with TCM consistently present microvasculature dysfunction findings^[33]. The characteristics of microvasculature dysfunction after acute psychological stress in patients with TCM include abnormality of endothelium-dependent vasodilation, excessive vasoconstriction, and impairment of myocardial perfusion^[34]. Uchida *et al*^[35] reported that extensive endothelial cell apoptosis was observed by myocardial biopsy. According to another report, increased susceptibility to ergonovine or acetylcholine followed by large vessel spasm, similar to vasospastic angina, may contribute to transient LV dysfunction^[36].

However, because only 30% of patients showed the characteristics of vasospasm in a challenge test, this theory was ruled out^[37,38]. Afonso *et al*^[39] demonstrated that circulatory disturbance, indicating coronary microvascular dysfunction was found on a myocardial contrast echocardiography and the epicardial coronary arteries were normal.

Myocardial biopsy of patients with TCM showed regions with contraction band necrosis, inflammatory cell infiltration, and localized fibrosis^[40]. These changes were caused by direct catecholamine toxicity on cardiac muscle cells^[41]. Morel *et al*^[42] found that C-reactive protein levels and white blood cell counts increased with the increase in norepinephrine levels in patients with TCM and inferred that catecholamines produced more systemic inflammation *via* the induction of proinflammatory cytokines such as tumor necrosis factor- α and interleukin-6. Several studies have pointed out that the remarkable myocardial edema, observed on cardiac MRI, occurs despite normal perfusion, which provides further evidence to support the inflammation theory^[43,44]. Ueyama *et al*^[45] examined restraint stress in rats with TCM and reported that heme oxygenase 1 (HO-1) levels, a marker of oxidative stress that has cardioprotective properties, was increased significantly. Macrophages play an important main role in oxidative stress induction and expression of β - and α -adrenergic receptors. As a result of pretreatment with

β - and α -antagonists, HO-1 expression and its altering gene expression, decreased.

RISK FACTORS

Lack of estrogen

More than 90% of patients with TCM are postmenopausal women. In fact, in a study to investigate if hormone replacement therapy had an effect on TCM, the authors concluded that none of the 31 patients with TCM received estrogen replacement therapy^[46]. Moreover, Ueyama *et al.*^[47] demonstrated that the decrease in LV function was greater in ovariectomized rats subjected to restraint stress than in rats receiving estradiol supplementation. The myocytes are known to express estrogen receptor- α and estrogen receptor- β . According to Ueyama *et al.*^[47], estrogen enhanced transcription of cardioprotective factors such as heat shock protein and atrial natriuretic peptide, and in turn, protected against the toxic effects of catecholamines, calcium overload and reduced oxidative stress^[48].

Emotional or physical stress inducers

A study reported on the prevalence of mood disorders and use of antidepressants in patients with TCM^[28]. When patients with depressive disorders experienced a stressful event, vagus nerve tension was decreased and response to adrenal medullary hormone was increased, which may be relevant to the cause of the disease^[49]. Further, some patients with depression showed very high noradrenaline extravasation^[50].

Genetic factors

Certain polymorphisms of α - and β -adrenergic receptors are associated with neurogenic stunned myocardium that occurs as symptom of subarachnoid hemorrhage and has overlapping pathophysiology with TCM^[51]. Although adrenoceptor polymorphisms have not yet been identified in patients with TCM, patients with this disease showed L41Q polymorphism of G protein coupled receptor kinase (GRK5) more frequently compared with the control group^[52]. L41Q polymorphism of GRK5 responds to catecholamine stimulation and attenuates the response of β -adrenergic receptors. Under catecholamine stimulation, balloon dilation of the ventricle may occur either by negative inotropic effect by β -receptor decoupling or ischemia because of an imbalance between α 1-adrenergic coronary artery vasoconstriction and β -adrenergic vasodilation. These reports suggest the very interesting possibility that the susceptibility to TCM in individuals may be partially related to genetic factors.

TREATMENT

Treatment of TCM during the acute phase is mainly symptomatic treatment. Intra-aortic balloon pump equipment is required for hemodynamically unstable patients in addition to cardiopulmonary circulatory support and continuous veno-venous hemofiltration^[53-55]. There is

controversy on the use of cardiac stimulants because of increased circulating catecholamines^[56]. However, cardiac stimulants are used in 20%-40% of patients with TCM^[2,57]. Levosimendan may be beneficial because of its inotropic action and vasodilator effect^[30,58]. Usage of anticoagulants may be considered at least until systolic function is recovered.

For patients with severe LV outflow tract obstruction with hemodynamic compromise, treatment with a β -blocker or α -adrenoceptor agonist such as phenylephrine and volume expansion should be considered. Calcium channel blockers can be used to decrease LV outflow tract pressure gradient. It is of utmost importance to avoid treatment with nitrites or inotropic drugs in these cases^[59-63]. For patients with suspected vasospasm, the use of calcium channel blockers such as verapamil or diltiazem is suggested^[64].

Hemodynamically stable patients are often treated with diuretics, angiotensin-converting enzyme (ACE) inhibitors and β -blockers. To reduce the risk of thromboembolism, patients with loss of motion of the LV apex should be treated with anticoagulant therapy until the contractility of the apex is improved unless there is a definite contraindication.

There is no consensus regarding long-term management of TCM, although it is reasonable to treat patients with β -blockers and ACE inhibitors during the ventricular recovery period. However, no data support the continuous use of these drugs for the prevention of TCM recurrence or improvement of survival rate. After LV function normalizes, physicians may consider discontinuation of these drugs.

PROGNOSIS AND RECURRENCE

Patients with TCM usually have a good prognosis, and almost perfect recovery is observed in 96% of the cases^[65]. Mortality rate in hospital vary at one to two percent^[18,66]. TCM was formerly thought to follow a relatively benign course. However, Sharkey *et al.*^[18] described that approximately 5% of TCM patients experienced cardiac arrest. While their long-term survival rate is the same as that in healthy subjects, patients with TCM have a greater risk of death at the time of initial onset^[65]. Elesber *et al.*^[65] reported that the most frequent chief complaint was chest pain (30%) and that recurrence of the symptom occurred in 11% of patients with TCM after a 4-year follow-up. Some studies have been conducted to assess prognostic indicators such as ECG findings, signs of thrombolysis in myocardial infarction, grade of myocardial perfusion, and N-terminal pro-brain natriuretic peptide level. However, a definite outcome marker has not been established^[66-68].

CONCLUSION

A lot of attention has been focused on TCM recently and this entity has been characterized as a transient LV dysfunction with rapid recovery generally induced by a stressful emotional or physical event. The number of

TCM cases continues to increase. Because of close resemblance of its presentation and clinical course to acute myocardial infarction, we believe that TCM should be included in one of the differential diagnosis for acute myocardial infarction. Although the cause of this disease has not been completely understood to date, some promising hypotheses have been suggested. The occurrence of this disease is attributed to the large-scale production of catecholamines that causes myocardial hypokinesia *via* direct cardiomyocyte toxicity and induction of coronary microvascular dysfunction. Further, the high prevalence of TCM in postmenopausal women suggests an important role of estrogen for myocardial protection. Another hypothesis includes oxidative/inflammatory stress-induced myocardial dysfunction. Although the treatment of TCM remains controversial, adrenergic blockade is suggested as a reasonable therapy based on the presumptive pathophysiology of TCM.

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WJC 6th Anniversary Special Issues (5): Myocardial infarction

Stem cell mechanisms during left ventricular remodeling post-myocardial infarction: Repair and regeneration

Rogelio Zamilpa, Mary M Navarro, Iris Flores, Sy Griffey

Rogelio Zamilpa, Mary M Navarro, Iris Flores, Sy Griffey, StemBioSys Inc., San Antonio, TX 78249, United States
Rogelio Zamilpa, Department of Comprehensive Dentistry, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, United States

Author contributions: Zamilpa R wrote the review; Navarro MM assisted with the tables, figures, revisions, and literature search; Flores I assisted with tables, figures, revisions, and literature search; Griffey S wrote review.

Correspondence to: Rogelio Zamilpa, PhD, StemBioSys Inc., 12500 Network Boulevard, Suite 105, San Antonio, TX 78249-3308, United States. zamilpa@uthscsa.edu

Telephone: +1-210-8779323 Fax: +1-210-8779323

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Core tip: Stem cell (SC)-based therapies hold promise to improve damaged myocardium repair and regeneration and thereby restore normal tissue function post-MI. In addition to the potential of SCs to regenerate myocardium, intrinsic properties of SCs such as their ability to home to areas of tissue damage make them an attractive tool for drug delivery. SCs, specifically mesenchymal stem cells, secrete multiple factors that can act in an autocrine and paracrine manner to regulate cell activation, recruitment, and survival during myocardium repair and regeneration.

Abstract

Post-myocardial infarction (MI), the left ventricle (LV) undergoes a series of events collectively referred to as remodeling. As a result, damaged myocardium is replaced with fibrotic tissue consequently leading to contractile dysfunction and ultimately heart failure. LV remodeling post-MI includes inflammatory, fibrotic, and neovascularization responses that involve regulated cell recruitment and function. Stem cells (SCs) have been transplanted post-MI for treatment of LV remodeling and shown to improve LV function by reduction in scar tissue formation in humans and animal models of MI. The promising results obtained from the application of SCs post-MI have sparked a massive effort to identify the optimal SC for regeneration of cardiomyocytes and the paradigm for clinical applications. Although SC transplantations are generally associated with new tissue formation, SCs also secrete cytokines, chemokines and growth factors that robustly regulate cell behavior in a paracrine fashion during the remodeling process. In this review, the different types of SCs used for cardiomyogenesis, markers of differentiation, paracrine factor secretion, and strategies for cell recruitment and

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INTRODUCTION

In the United States alone, it is estimated that a myocardial infarction (MI) occurs every 35 s and approximately 20% of patients that experience a first-MI develop heart failure (HF) within 5 years^[1]. An MI is consensually defined as the death of cardiomyocytes after a prolonged period of ischemia causing a progressive decline in cardiac function that ultimately results in HF^[2]. Although the mortality associated with acute MI continues to decline as a result of revascularization, the morbidity and mortality

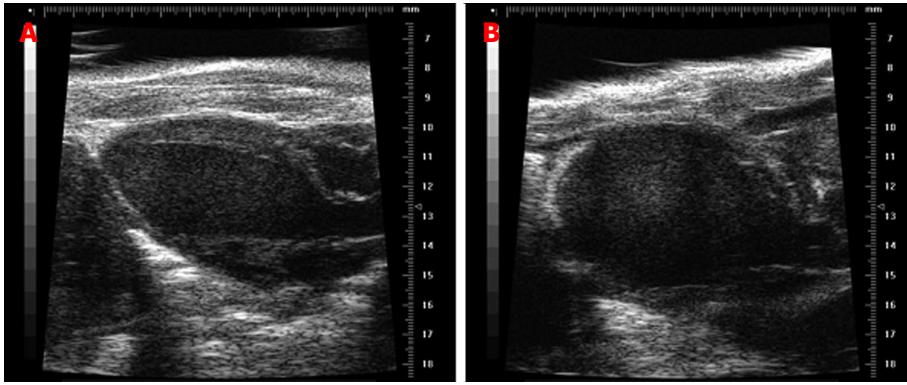


Figure 1 During the course of left ventricular remodeling, (A) the normal elliptical shape of the left ventricular changes to spherical (B) as illustrated by the echocardiograms of the mouse permanent ligation myocardial infarction model. Image A was recorded at baseline and image B was recorded at day 7 post-myocardial infarction.

caused by HF is on the rise^[3,4].

Current post-MI pharmacological therapies such as ACE inhibitors and beta-blockers improve cardiac repair and slow down the progression to HF. However, the growing interest in stem cell (SC) therapies which not only promote repair but also hold promise to regenerate damaged myocardium has sparked a tremendous effort aimed at the development of an effective paradigm for ventricular remodeling post-MI. The possibility that SC therapies can restore cardiac function post-MI and increased evidence that the heart contains resident SCs niches has also contributed to this growing interest^[5-7].

Post-MI, the LV undergoes a remodeling process that results in the replacement of damaged myocardium with a collagen scar^[8-10]. During the remodeling process, the normal elliptical shape of the LV (Figure 1A) changes to spherical (Figure 1B) as illustrated by the echocardiogram of the murine heart following MI induced by permanent ligation of the left anterior descending coronary artery. Along with the architectural and structural changes, LV contractile function declines^[10].

The magnitude of LV contractile dysfunction is dependent on the extent of the infarct and the wound healing response that follows which includes cardiomyocyte death, inflammatory response, granulation tissue synthesis and granulation tissue maturation and remodeling. Historically, the use of stem cells has automatically been associated with direct replacement of dead cardiomyocytes; however, more recent research has indicated that stem cells possess intricate properties that can regulate other aspects of myocardium repair post-MI. In this review we will focus on the application of stem cells as a therapeutic tool for treatment of myocardial damage post-acute MI and discuss the role of stem cells during cardiac repair and regeneration.

OVERVIEW OF STEM CELL ROLES IN REPAIR AND REGENERATION

SCs are sophisticated cells with multifunctional properties that can orchestrate the wound healing process post-MI

leading to restoration of normal tissue function (Figure 2). One of these properties is the ability to home to areas of injury which has led to the investigation of stem cells for targeted drug delivery^[11-13]. Post-MI, SC transplantations have been shown to rescue apoptotic cardiomyocytes and give rise to mature cardiomyocytes through cell fusion^[14,15]. In addition, multiple SC types have the capability of differentiating into functional cardiomyocytes which suggest that SCs can be used to replace necrotic or apoptotic cells post-MI. Further, SC transplantations have been shown to regulate the inflammatory response, reduce scarring, and promote angiogenesis through the paracrine effects, all of which lead to improved cardiac function in humans and animal models post-MI.

Cell fusion

A major mechanism of action of SC transplantation post-MI that contributes to cardiac repair and regeneration is achieved through cell fusion. Using a combination of *in vitro* cell culture models and *in vivo* animal models of MI, fusion rates of SCs with injured cardiomyocytes were shown to significantly increase^[14,15]. As a result, there was a decrease in cardiomyocyte apoptosis and an increase in the generation of mature cardiomyocytes^[14-16]. Interestingly, inhibition of apoptosis was also achieved through paracrine effects using *in vitro* co-culture models through activation of the anti-apoptotic AKT/PKB pathway^[15,16].

Replacement of dead cardiomyocytes

One of the primary goals of SC therapies post-MI is the replacement of dead cardiomyocytes. The current challenge in this regard is to identify the optimal SC for cardiomyocyte replacement. SCs are broadly classified based on their tissue of origin including embryonic *vs* adult, hematopoietic *vs* non-hematopoietic, and are further subcategorized by their differentiation potential. Stem cell differentiation potential is their ability to differentiate into specialized cells. By definition, a SC is not committed to one specific lineage and must therefore be given the appropriate differentiation signals if the paradigm calls for a cardiac progenitor or cardiomyocyte-differentiated cell.



Figure 2 Stem cells possess multifunctional properties to promote damaged myocardium repair and regeneration post-myocardial infarction. As illustrated by this model, stem cells have a tremendous ability to home to sites of injury, fuse with injured cells, inhibit cardiomyocyte apoptosis, replace dead cardiomyocytes, as well as secrete paracrine factors to regulate the inflammatory response, fibrosis, and neovascularization post-myocardial infarction. LV: Left ventricle; SC: Stem cell; MI: Myocardial infarction.

In Table 1, SCs that have been differentiated into a cardiogenic lineage and the methods of differentiation are listed.

Embryonic stem cells (ESCs) have been differentiated into cardiomyocytes *in vitro* and *in vivo*. Expression of transcription factors GATA-4, myocyte-specific enhancer factors (MEF) 1 and 2C, and Nkx2.5 are commonly used for assessment of cardiomyocyte differentiation. Other factors such as atrial natriuretic factor, myosin light chain (MLC)-2v, myosin heavy chain (MHC), and phospholamban have also been used^[16-18].

Human induced pluripotent stem (iPS) cells from various sources, including reprogrammed cardiac fibroblasts, have been differentiated into functional cardiomyocytes^[19-23]. Early cardiac lineage differentiation markers include GATA-4, GATA6, Nkx2-5, the T-box 5 (Tbx5), insulin gene enhancer protein-1 (Isl1), and LIM homeodomain transcription factor^[20].

To date, the most commonly used SCs for cardiac tissue regeneration have been derived from adult bone marrow. In 2001, Orlic *et al*^[24] demonstrated that c-kit positive cells derived from bone marrow were able to generate de novo myocardium indicating that these cells might be ideal for treatment post-MI. Expansion of this study has demonstrated that bone marrow hematopoietic SCs give rise to cardiomyocytes through cell fusion rather than differentiation. Expression of α -actin, cardiac troponin T, and connexin-43 has been used for cardiac lineage differentiation^[25]. In addition to c-kit positive cells, the bone marrow contains fibroblast-like, mesenchymal stromal cells (MSCs) also known as mesenchymal stem cells^[26,27].

Studies using bone marrow MSCs have demonstrated that transplanted MSCs mobilize from the bone marrow into the ischemic myocardium post-MI. Consequently, these cells differentiate into cardiomyocytes suggesting that these cells play important roles in repair and regeneration post-MI^[27-29]. Expression of α -actin, cardiac titin, cardiac troponin T, desmin, MHC, MEF 2A and 2D, and phospholamban have been used as markers for MSC cardiomyocyte differentiation^[27,30,31].

Human-derived adipose MSCs, amniotic fluid SCs, umbilical cord blood hematopoietic cells and MSCs, and Wharton's Jelly MSCs have also been differentiated to cardiomyocytes. Expression of α -actin, cardiac troponin I, GATA4, MHC, N-cadherin, Nkx2.5, and Tbx5 have been used for cardiac lineage differentiation characterization^[32-38].

Interestingly, cardiac tissue homeostasis and regenerative potential has been shown to involve resident cardiac SCs and progenitor cells which have been isolated and expanded from adult human and mouse heart tissue biopsies^[39-41]. At least four different types of resident cardiac SCs have been isolated and shown to differentiate into cardiomyocytes^[41-47]. Interestingly, three of the four types of resident cardiac SCs identified so far have the ability to form cardiospheres (CS)^[41,47]. α -actin and MEF2C are expressed by cardiomyocyte progenitors and developing cardiomyocytes. In addition to cardiosphere-derived cells, cardiac side population cells isolated from neonatal rat hearts have also been differentiated into beating cardiomyocytes by treatment with oxytocin or trichostatin A. *In vivo*, cardiac side specific cells demonstrated a superb

Table 1 Stem cells differentiated into cardiomyocytes

Cell type	Method of differentiation	Ref.
ESCs	EB-mediated differentiation	[17,18]
iPS	Transdifferentiation of iPS cell factor-based reprogrammed cardiac fibroblasts using EB-based method + transwell CM co-culture system	[19]
	Direct reprogramming of cardiac fibroblasts <i>in vivo</i> by local delivery of GMT	[21]
	Suspension EB-mediated differentiation of reprogrammed adult fibroblasts	[22,23]
Bone marrow MSC	<i>In vitro</i> differentiation induced by treatment with 5-azacytidine	[27,28]
	<i>In vivo</i> differentiation of stem cells transplanted and mobilized to damaged myocardium	[29]
	<i>In vivo</i> differentiation of stem cells engrafted into the myocardium	[30]
	Differentiation using a cardiomyogenic differentiation medium containing insulin, DMSO, and ascorbic acid	[31]
Adipose-derived MSC	Co-culture in direct contact with contracting cardiomyocytes	[37]
	DMSO at 0.1% for 48 h	[38]
Amniotic fluid SCs	<i>In vivo</i> differentiation of cells transplanted into myocardium	[33]
	<i>In vitro</i> differentiation through EB formation	[35]
Umbilical cord blood SCs	Co-culture with primary rat neonatal ventricular myocytes	[32]
	Co-culture with mouse neonatal cardiomyocytes	[34]
Wharton's Jelly MSCs	<i>In vitro</i> differentiation induced by treatment with 5-azacytidine or by culture in cardiomyocyte CM	[36]
CS	Co-culture with neonatal rat cardiomyocytes	[41]
CSP	Treatment with oxytocin or trichostatin A	[48]

ESC: Embryonic stem cell; EB: Embryoid body; iPS: Induced pluripotent stem cells; SCs: Stem cells; CM: Conditioned medium; GMT: Gata4, Mef2c, and Tbx5; MSC: Mesenchymal stem cell; DMSO: Dimethylsulfoxide; CS: Cardiospheres; CSP: Cardiac side population.

ability to home to injured heart and differentiate into cardiomyocytes. Expression of cardiac transcription factors GATA-4, Nkx2.5 and MEF 2C as well as contractile proteins MHC and MLC-2v have been used for SP cell cardiomyocyte differentiation^[48].

Regulation of the inflammation

In addition to the ability of SCs to potentially replace dead cardiomyocytes, SCs provide a rich source of cytokines and growth factors that can act in an autocrine, paracrine, or endocrine fashion to regulate cell behavior during the inflammatory reaction post-MI^[49].

The inflammatory response that follows an MI is necessary and plays a crucial role in proper healing and ventricular remodeling. Post-MI, myocardial necrosis initiates an inflammatory response that includes a cascade of cytokines and chemokines followed by recruitment of neutrophils and macrophages^[50,51]. As summarized by Frangogiannis *et al.*^[51], the inflammatory reaction clears

the damaged myocardium of cellular and matrix debris and activates the reparative process^[51]. A prolonged inflammatory reaction leads to adverse remodeling and ventricular dysfunction due to untimely resolution of the acute inflammatory response, increased cardiomyocyte loss and resultant negative downstream effects to extracellular matrix (ECM) metabolism and neovascularization^[50].

The most commonly used SC for transplantations post-MI are bone marrow-derived MSCs. The paracrine effects of MSCs have received far more recognition than their ability to replace dead cardiomyocytes. One of the therapeutic goals post-MI is to minimize cardiomyocyte loss. Transplantation of bone marrow MSCs has been shown to reduce cardiomyocyte loss through activation of the cell survival gene Akt^[52]. Further, other anti-apoptotic effects of MSCs are postulated to include inhibition of nuclear factor κ B (NF- κ B) activity, reduced production of tumor necrosis factor α (TNF- α) and interleukin 6 (IL-6) as well as increased expression of IL-10^[53-55].

As part of their involvement in the inflammatory response post-MI, polymorphonuclear granulocytes (PMNs; neutrophils) leave the circulation, infiltrate into the injured myocardium, secrete proteolytic enzymes and reactive oxygen species, and clear cellular and ECM debris^[56,57]. Increased production of IL-6 by MSCs has been shown to prevent apoptosis by activated neutrophils thereby increasing the lifespan of neutrophils through STAT3 transcription factors^[58-60]. In addition, the increased production of IL-6 regulates neutrophil activation by attenuation of the respiratory burst^[59,60].

Macrophages in the injured myocardium undergo a biphasic activation that begins with a pro-inflammatory phase (also known as M1 or classically activated) that is followed by an overlapping anti-inflammatory phase (also known as M2 or alternatively activated)^[61,62]. The macrophage polarization switch from M1 to M2 is a key event in myocardium repair^[51,63]. MSC transplantations post-MI increase the number of M2 macrophages^[64]. While the mechanism is still unclear, it is likely mediated through paracrine effects that include CCL2, galectin-1, interferon- γ , IL-1 β , indoleamine-2,3-dioxygenase, IL-4, IL-6, IL-10, IL-13, prostaglandin-E2, TNF- α , NF- κ B, nitric oxide, heme oxygenase-1, hepatocyte growth factor, transforming growth factor-b1, and Human Leukocyte Antigen-G5^[53,64,65].

MSC paracrine factors have also been shown to suppress T cell, natural killer cell, and B cell proliferation and attenuate the maturation of dendritic cells through paracrine factors as listed in Table 2^[60,66-68].

Regulation of fibrosis

Post-MI, necrotic cardiomyocytes are replaced with a fibrous scar. The extent of damaged tissue degradation and subsequent production of a provisional ECM affects scar thickness which in turn influences contractility of the surrounding myocardium. An increased degradation of ECM results in wall thinning and the development of aneurysms and LV rupture while an increased production

Table 2 Stem cell trophic factors

Factor	Outcome	Ref.
↑Akt	Reduction cardiomyocyte loss	[52]
↓NF-κβ	Anti-apoptotic effects	[53-55]
↓TNF-α	Anti-apoptotic effects	[53-55]
↓IL-6	Anti-apoptotic effects	[53-55]
↑IL-10	Anti-apoptotic effects	[53-55]
↑IL-6	Prevention activated neutrophil apoptosis <i>via</i> Stat3; regulation of neutrophil activation	[56-60]
↑IL-10, ↑TNF-α, and ↑IL-6	Macrophage M2 polarization	[53,61-65]
↓Collagen I and III, ↓TIMP-1 and ↓TGF-β	Reduction in fibrosis and scar size	[55,69-76]
↑VEGF	Promote angiogenesis; improved contractile function	[77-86]
↑IL-6	DC maturation inhibition	[60,66-68]
↑IDO and ↑PGE2	Reduced T cell activation	[60,66-68]
↑IDO and ↑PGE2	Decreased NK proliferation	[60]
Factor to be identified	B-Cell arrest	[60]

Akt: Serine/threonine kinase; NF-κβ: Nuclear factor κβ; TNF-α: Tumor necrosis factor α; IL-6: Interleukin 6; IL-10: Interleukin 10; TIMP-1: Tissue inhibitor of metalloproteinase 1; TGF-β: Transforming growth factor β; VEGF: Vascular endothelial growth factor; IDO: Indoleamine 2,3-dioxygenase; DC: Dendritic cell; NK: Natural killer cell; PGE2: Prostaglandin E2.

of ECM results in fibrosis and can predispose the LV to HF^[69]. Interestingly, SC transplantations post-MI have been shown to regulate scar formation post-MI and improve ventricular function.

Transplantation of beating cardiomyocytes produced *in vitro* from ESCs has been shown to attenuate scar thinning and increase fractional shortening post-MI^[70]. iPS cell therapy in the mouse permanent ligation model has also been shown to reduce wall thinning post-MI^[71]. Additionally, MSC transplantations have been shown to reduce fibrosis and scar size^[55,72-74]. Studies by Xu and colleagues demonstrated that MSC transplantations in rats post-MI regulate LV remodeling by decreasing mRNA expression and protein levels of TGF-β, type I and type III collagens, and tissue inhibitor of metalloproteinase (TIMP)-1^[75]. Interestingly, in sheep, MSC progenitor cell-injections into the border zone altered collagen dynamics in a cell concentration-dependent manner as a result of spatial changes in matrix metalloproteinases (MMPs) and TIMPs. MMPs -1, -2, -3, -7, -9, -13, MT1-MMP, and TIMPs -1, -2, -4 were differentially altered in the remote, border zone, and infarct zones post-injection^[76].

Regulation of angiogenesis

Angiogenesis is essential for myocardium repair and scar formation post-MI, and paracrine factors released following SC transplantations promote angiogenesis^[77,78]. MSCs that engraft after transplantation post MI have been shown to express endothelial cell markers^[79,80]. Consistent with these findings, MSCs have also been shown to secrete significantly elevated levels of vascular endothelial growth factor (VEGF). Concomitantly, capillary density increases in the infarct region contributing to improved regional and contractile function^[81-83]. It is important to

note that MSCs, preconditioned under hypoxic conditions, have an enhanced capacity to stimulate vascularization compared to MSCs cultured under normoxic conditions due to increased expression of VEGF, angiopoietin-1, and survival post-transplantation^[84-86].

Stem cell recruitment and delivery strategies

Several strategies have been used for SC therapeutic applications post-MI. These include cell infusion intravenously, intramyocardial injections, intracoronary applications, endocardial applications, and engineered delivery methods such as cardiac patches^[87,88]. For SC recruitment, identification of chemoattractants that are responsible for SCs homing to damaged myocardium has shown an improvement in repair and ventricular function post-MI. Overexpression of stromal cell-derived factor-1 by transfected fibroblasts injected into the peri-infarct zone increased hematopoietic SC homing and improved fractional shortening in the rat MI model^[89]. Monocyte chemotactic protein-3 also delivered in a similar fashion *via* transfected fibroblasts was shown to increase MSC engraftment. Although no significant regeneration of cardiomyocytes was observed, fractional shortening increased and LV end diastolic dimensions decreased^[90]. In the porcine MI model, the combination of insulin growth factor-1 and hepatocyte growth factor activated endogenous cardiac SCs resulting in regeneration of cardiomyocytes and angiogenesis as well as improved cardiac function^[91]. Interestingly, thymosin β4 has also been shown to play important roles in epicardial progenitor cell mobilization in the mouse heart for neovascularization^[92,93].

For delivery, biological and synthetic scaffolds used as vehicles for SC transplantations have shown improvement in cell survival, engraftment and cardiomyogenesis. In the rat MI model, transplanted cardiac SCs using nano-topographical hydrogel patches that mimicked the native cardiac ECM improved cell integration, retention and myocardium regeneration^[94]. Similarly, cardiac patches containing adipose stromal vascular cells increased coronary blood flow and significantly improved ejection fraction post-MI^[95,96]. The combination of a hydrogel patch with encapsulation of MSCs, as designed by Levit and colleagues, improves cell survival and takes full advantage of MSC paracrine factors. In addition to significantly reduced scar size, delivery of encapsulated MSCs increased peri-infarct microvasculature and improved ejection fraction in the rat MI model^[97].

LIMITATIONS ASSOCIATED WITH SC TRANSPLANTATIONS POST-MI

Although numerous studies in humans and animal models have demonstrated that SC transplantations post-MI are safe and can improve cardiac healing and function, several common limitations associated with SC transplantations have been reported. The most common issues with SC transplantations for ventricular remodeling post-MI include reduced cell survival and engraftment which

Table 3 Recently published clinical trials of stem cell therapies for acute treatment post-myocardial infarction

	Clinical trial	Outcome	Ref.
2010	Influence of bone marrow stem cells on left ventricular perfusion and ejection fraction in patients with acute myocardial infarction of anterior wall: Randomized clinical trial	Slight improvement of myocardial perfusion	[109]
2011	HEBE trial	No significant improvement on regional or global function	[110]
2011	Late TIME trial	No improvement on global or regional function at 6 mo	[111]
2012	Stem cell treatment for acute myocardial infarction	Reduced LVESV, LVEDV, and infarct size	[109]
2012	CADUCEUS trial	Reduced scar mass, increased myocardium viability, regional contractility and wall thickness	[112]
2012	Enhanced mobilization of the bone marrow-derived circulating progenitor cells by intracoronary freshly isolated bone marrow cells transplantation in patients with acute myocardial infarction	Feasibility, safety, and improvement on recovery of LV contractility	[113]
2013	The C-CURE trial	Feasibility, safety and improved LV ejection fraction	[114]

LV: Left ventricular; LVESV: Left ventricular end systolic volume; LVEDV: Left ventricular end diastolic volumes; CADUCEUS: CARDiosphere-derived autologous stem cells to reverse ventricular dysfunction; C-Cure: Cardiopoietic stem cell therapy in heart failure.

ultimately result in diminished cardiac regeneration and limited functional benefits. In human clinical trials, 3.2% of bone marrow SCs remained 24 h post-infusion, and in agreement with this outcome, other studies report less than 10% SC retention in human and animal studies^[97-102]. Further, SCs that do engraft may differentiate into other lineages such as endothelial cells and fibroblasts rather than cardiomyocytes^[103-105]. With regard to delivery methods, intravenous infusions may have decreased efficacy due to entrapment of cells in non-target tissues and organs such as bone marrow, lungs, liver and spleen^[106,107]. Similarly, intracoronary and intramyocardial delivered cell retention is also limited and may reduce the efficacy of the transplanted cells due to the hostile milieu of the damaged heart^[108,109]. Other reported issues with SC delivery methods include the potential for microembolism formation (intravenously and intracoronary), and the potential to induce arrhythmias (intracoronary and intramyocardial)^[87,88].

Translation from bench to bedside

The ultimate goal of SC applications is the translation of what has been learned in the laboratory to the production of safe and effective therapies for attenuation of adverse LV remodeling. In Table 3, the results from the most recently published clinical trials of SC therapies in treatment of myocardial damage post-acute MI are listed. In addition to the feasibility of cell delivery, the safety associated with SC transplantations continues to evolve in clinical trials. Conversely, common issues such as standardization of methodology (including cell dosing, cell product formulation, and timing of transplantation) and the innate heterogeneity of study populations which include other clinical factors such as advanced aging and diabetes hinder interpretation of trial outcomes resulting in the need for a larger-scale study^[100-114]. On this front, it is very encouraging to see a significant increase in the number of clinical trials being performed across the globe. The Alliance for Regenerative Medicine annual report for 2012-2013 indicates there were 326 industry-sponsored cell therapy trials ongoing in early 2013. The report fur-

ther indicates that the number of early to mid-stage cell therapy trials in cardiovascular-related diseases ranks second only to cell therapy studies involving cancer^[115].

A more specific review of acute MI clinical trials reveals that there were 36 open studies registered under “acute myocardial infarction and stem cells”. For congestive heart disease clinical trials the search revealed that there were 48 open studies registered under “congestive heart failure and stem cells” as of the writing of this review. Of these studies, there were 16 listed in phase 1 trials, 25 phase 2 trials, and 9 phase 3 trials (note that some studies are listed in overlapping phases). The majority of these studies are being conducted in the European Union and the United States with 15 and 12 registered studies, respectively^[116].

CONCLUSION

The results from post-MI SC transplantations in animal models and humans have provided promising results in reducing scar formation and improved LV function which are achieved primarily through paracrine effects. While a great deal of information has been obtained in the past two decades on the roles SCs play in the post-MI setting, additional studies are needed to improve the efficacy of stem cell transplantations post-MI. Further, a consensus on the best time to initiate treatment, dosage, and delivery method is needed.

In summary, we have reviewed the current literature on the roles SCs play during LV remodeling post-MI. This evaluation includes different types of SCs with cardiomyogenic potential, markers of differentiation, trophic effects for the inflammatory, fibrotic and vascularization responses as well as strategies for cell homing and delivery post-MI.

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Chronic total occlusion: To treat or not to treat

Alfredo Bardají, Judit Rodríguez-López, Mauricio Torres-Sánchez

Alfredo Bardají, Judit Rodríguez-López, Mauricio Torres-Sánchez, Servicio de Cardiología, Hospital Universitario de Tarragona Joan XXIII, Universitat Rovira Virgili, 3007 Tarragona, España

Author contributions: Bardají A, Rodríguez-López J and Torres-Sánchez M performed research; Bardají A wrote the paper.

Correspondence to: Alfredo Bardají, MD, PhD, FESC, Servicio de Cardiología, Hospital Universitario de Tarragona Joan XXIII, Universitat Rovira Virgili, Calle Dr Mallafré Guasch 4, 43007 Tarragona, España. alfredo.bardaji@urv.cat

Telephone: +34-97-7295834 Fax: +34-97-7295859

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this review, we will consider the available information supporting percutaneous treatment for chronic occlusions, as well as the areas of uncertainty where more research projects are required.

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Key words: Chronic total occlusion; Percutaneous coronary intervention

Core tip: This is a critical review about the available information supporting percutaneous treatment for chronic occlusions, as well as the areas of uncertainty where more research projects are required.

Abstract

Over the last two decades, there has been increasing interest in new techniques for the percutaneous treatment of coronary chronic total occlusions (CTO), which have a success rate that is much higher than that of a few years ago. The rise in percutaneous treatment for these lesions is due to its ability to improve the symptoms and prognosis of patients in the chronic and stable phase of coronary disease. Current data suggest that successful percutaneous coronary intervention for CTO is associated with improvement in patient symptoms, quality of life, left ventricular function, and survival, compared with those with unsuccessful CTO PCI. However, all the scientific evidence supporting this treatment comes from observational studies, and no randomized study comparing percutaneous treatment with medical treatment has yet been published. A major limitation of these studies is their observational design, with limited information with regard to potential baseline differences between the successful vs unsuccessful cohorts. Pending randomized studies, patients should be selected very carefully, especially if they are asymptomatic or very few symptoms, and the benefits obtained in terms of complications during the procedure, the quality of life obtained and further ischemic events avoided should be evaluated systematically. In

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INTRODUCTION

Chronic total occlusions (CTO) are considered to be 100% coronary lesions, of more than 3 mo evolution^[1]. They are therefore always found in stable chronic patients, with varying levels of symptoms. After the culprit artery has been treated, patients with acute coronary syndrome may occasionally also have a chronic occlusion in another artery that was not responsible for the acute event, and is therefore considered a CTO.

DEFINITION AND INCIDENCE

The prevalence of CTO in patients undergoing coronary angiography varies, ranging between 18% and 52% depending on the clinical profile of the patient being examined^[2-7] (Table 1). Although revascularization surgery is the most frequent treatment, clinicians and invasive cardi-

Table 1 Chronic total occlusion prevalence, location and treatment applied in different studies *n* (%)

Ref.	Type of study	Population	CTO prevalence	CTO location			Medical treatment	PCI	CABG
				RCA	LAD	LCA			
Kahn <i>et al</i> ^[2] , 1993	Retrospective	333	101 (35)	58%	18%	24%	-	-	-
Christofferson <i>et al</i> ^[3] , 2005	Retrospective	Coronary disease (stenoses \geq 50%)	1612 (25)	49.4%	22%	28.60%	49%	11%	40%
		Underwent coronarography because of suspected CD							
		3087	1612 (52)						
Srinivas <i>et al</i> ^[4] , 2002	Retrospective	Coronary disease (stenoses \geq 70%)	1761					14.50%	-
		Multivessel disease							
Yamamoto <i>et al</i> ^[5] , 2013	Prospective	15263	2491 (19)	44.9%	41.10%	28.50%	-	61.18%	-
Fefer <i>et al</i> ^[6] , 2012	Prospective	First revascularization procedure	14439						
		Underwent coronariography because of suspected CD	2630 (18.2)	46.9%	19.86%	15.43%	64%	10%	26%
Jeroudi <i>et al</i> ^[7] , 2013	Prospective	1015	319 (31.34)	-	-	-	19% (61)	50% (161)	30% (97)
		Coronary disease (stenoses \geq 50%)							

CTO: Chronic total occlusion; RCA: Right coronary artery; LAD: Left anterior descending; LCA: Left circumflex artery; PCI: Percutaneous coronary intervention; CABG: Coronary artery bypass graft.

ologists often consider the need and feasibility of percutaneous treatment for these lesions, based on symptoms and prognostic factors. However, as it is a common problem in all Cath Labs, the extensive variability between different centres is striking. For example, in North American centres^[4], with an incidence rate of CTO of between 29% and 33% in all the catheterizations performed, only between 6% and 9% of patients were treated percutaneously. However, in Japanese centres, with an incidence of 19% of CTO in all the catheterizations performed, 61.2% of all cases were treated percutaneously^[5]. There are also significant differences in the treatment of CTO within the same geographical area or healthcare system. For example, in the Canadian CTO registry^[6], some hospitals percutaneously treat 16% of their patients, while others only do so for 1%. These differences are very striking, and can only be justified by some generally ill-defined indications, as well as the technical difficulty that means that not all invasive cardiologists can or should deal with complex lesions of this type. However, there is another factor that also needs to be mentioned. Patients with CTO probably have a clinical profile that is different to that of patients with chronic coronary ischemic disease in general. There not only are differences in terms of greater severity of coronary disease, but also in terms of increased non-coronary comorbidity, such as a higher rates of prevalence of diabetes, peripheral arterial disease, heart failure and a history of strokes^[8]. The indications for percutaneous treatment of CTO are not well defined in the European guidelines for revascularization^[9], or in the guidelines for patients with stable chronic coronary disease^[11] (Table 2). The American guidelines on revascularization^[10,11] and chronic stable ischemic heart disease^[12] are also unclear as regards the indications for treatment of CTO. Only the American guidelines for the appropriate use of percutaneous coronary treatment^[13] contain a clear position on treatment that is appropriate, uncertain

or not indicated in CTO lesions (Table 3).

CTO TREATMENT IN PATIENTS WITH ANGINA

There should be no doubt that a treatment of a CTO affecting an ischemic myocardial area that causes symptoms such as angina should improve patients' symptoms, by providing a greater perfusion flow than that provided by collateral circulation, as a consequence of opening the occluded artery^[14]. However, this has been poorly studied and quantified in the medical literature. Very few studies have specifically evaluated the changes in the ischemic threshold and quality of life scales of symptomatic and asymptomatic patients with percutaneously treated CTO. In the FACTOR Trial (FlowCardia Approach to CTO Recanalization), 125 patients completed the Seattle Angina Questionnaire at baseline and one month after percutaneous coronary intervention^[15]. Successful treatment was associated in overall terms with an improvement in the frequency of angina, physical capacity and quality of life. However, this improvement was only observed in previously symptomatic patients but not in asymptomatic patients. In fact, this symptomatic improvement is similar to that obtained with percutaneous coronary intervention in the treatment of lesions without chronic total occlusion^[16].

TREATMENT OF CTO IN ISCHEMIC PATIENTS

Often no distinction is made between patients with angina and patients with myocardial ischemia when percutaneous treatment of CTO is indicated^[17]. However, these two concepts are different in our opinion, and should be clarified. In patients with angina (and therefore with

Table 2 Specific recommendations on the treatment of chronic total occlusion in the American and European Practice Guidelines

Society	Guideline	Specific recommendation on the treatment of CTO
EUROPEAN	2010 Guidelines of myocardial revascularization ^[9]	"Revascularization of CTO may be considered in the presence of angina or ischemia related to the corresponding territory"
	2013 ESC guidelines on the management of stable coronary artery disease ^[1]	"Revascularization needs to be discussed in patients with symptoms of occlusion or large ischemic areas"
AMERICAN	2011 ACCF/AHA Guideline for Coronary Artery Bypass Graft Surgery ^[10]	Not mentioned
	2011 ACCF/AHA/SCAI Guideline for Percutaneous Coronary Intervention ^[11]	Recommendation IIa. Evidence level B. PCI of a CTO in patients with appropriate clinical indications and suitable anatomy is reasonable when performed by operators with appropriate expertise "The decision to try PCI for a CTO (<i>vs</i> continued medical therapy or surgical revascularization) requires an individualized risk-benefit analysis encompassing clinical, angiographic, and technical considerations"
	2012 ACCF/AHA/ACP/AATS/PCNA/SCAI/STS Guideline for the Diagnosis and Management of Patients With Stable Ischemic Heart Disease ^[12]	Not mentioned

CTO: Chronic total occlusion; ACCF: American College of Cardiology Foundation; AHA: American Heart Association; SCAI: Society for Cardiovascular Angiography and Interventions; ACP: American College of Physicians; AATS: American Association for Thoracic Surgery; PCNA: Preventive Cardiovascular Nurses Association; STS: Society of Thoracic Surgeons; PCI: Percutaneous Coronary Intervention.

Table 3 Specific recommendations on the treatment of chronic total occlusion in the 2012 ACCF/SCAI/STS/AATS/ASNC/HFSA/SCCT Appropriate Use Criteria for Coronary Revascularization Focused Update^[14]

		ANGINA							
		Asymptomatic	I	II	III	IV			
Risk in the ischemia test	High	Uncertain	Appropriate	Appropriate	Appropriate	Appropriate	Max	Treatment level	
		Uncertain	Uncertain	Uncertain	Appropriate	Appropriate	Med		
		Uncertain	Uncertain	Uncertain	Appropriate	Appropriate	Min		
	Medium	Uncertain	Uncertain	Uncertain	Appropriate	Appropriate	Max		
		Inappropriate	Uncertain	Uncertain	Uncertain	Uncertain	Med		
		Inappropriate	Uncertain	Uncertain	Uncertain	Uncertain	Min		
	Low	Inappropriate	Inappropriate	Inappropriate	Uncertain	Uncertain	Max		
		Inappropriate	Inappropriate	Inappropriate	Inappropriate	Inappropriate	Med		
		Inappropriate	Inappropriate	Inappropriate	Inappropriate	Inappropriate	Min		

It shows the 45 possible scenarios depending on the risk of mortality based on the findings on ischemia tests, symptoms and level of treatment.

ischemia), the benefit of CTO treatment is for the symptoms and possibly the prognosis. However, as mentioned above, in patients with ischemia but without angina, the benefit is not symptomatic and can only be evaluated in prognostic terms. It is therefore important to determine whether patients with myocardial ischemia but who are asymptomatic benefit from percutaneous treatment of a CTO. The rationale for this approach is based on relatively early studies in which the improvement of ischemia provided by the revascularization obtained by an angioplasty, in both symptomatic patients^[18] and asymptomatic patients^[19], was associated with an improved prognosis. In the SWISSI II Trial^[19] in the late 1990s, on asymptomatic patients after myocardial infarction, with coronary disease in 1 or 2 vessels and inducible myocardial ischemia in an imaging stress test, coronary angioplasty significantly reduced coronary events during a long-term follow-up period. However, in this study, both the medical treatment, which was very limited, and the percutaneous treatment (the use of bare metal stents) were obviously different to those currently in use. More recent studies of

symptomatic patients with chronic coronary disease, frequently presenting a positive test for ischemia, have failed to show that percutaneous revascularization improves prognosis^[20], even in diabetic patients^[21]. In the COURAGE trial, the small benefit in terms of improved quality of life in percutaneously treated patients compared to those receiving medical therapy without revascularization disappeared after 36 mo follow-up^[22]. The data from the COURAGE trial substudy, with quantification of ischemia by a stress test with nuclear imaging, show that in patients with stable chronic ischemic heart disease, angioplasty provides a greater improvement in the ischemic area than medical treatment^[23]. However, this improvement in the ischemic area had no effect on the medium-term prognosis^[24]. A recent meta-analysis including all the randomized studies in patients with stable chronic ischaemic cardiopathy and proven myocardial ischemia concluded that percutaneous treatment does not affect rates of mortality, myocardial infarction, unplanned revascularization or angina compared to medical treatment alone^[25]. At present, the hypothesis that moderate to severe

Table 4 Findings on left ventricular ejection fraction and regional wall motion variations after percutaneous coronary intervention treatment of chronic total occlusion

	Type of study	Population	LVEF estimation	Follow up	Results				
					LVEF	Regional wall motion	Symptoms	Collateral function	Ventricular remodeling
1994-1995 Sirnes <i>et al</i> ^[30]	Prospective	95 CTOs treated with PCI	Ventriculography	Angiography 6 mo	LVEF increase (from 0.62 ± 0.13 to 0.67 ± 0.12) <i>P</i> < 0.001	Increase in regional radial shortening (from 0.279 ± 0.106 to 0.319 ± 0.107) <i>P</i> < 0.001	Improvement in angina class	Not mentioned	Not mentioned
1999-2003 Werner <i>et al</i> ^[31]	Prospective	126 CTOs treated with PCI	Ventriculography	Angiography	LVEF increase (from 0.60 ± 0.19 to 0.67 ± 0.16) <i>P</i> < 0.001	Increase in wall motion severity index (from -1.92 ± 1.32 to -1.30 ± 1.28) <i>P</i> < 0.001	Not mentioned	No changes in collateral function	Not mentioned
2008 Kirschbaum <i>et al</i> ^[32]	Prospective	21 CTOs treated with PCI	NMR	NMR 5 mo and 3 yr	LVEF increase (from 60% ± 9% to 63% ± 11%) <i>P</i> = 0.11	Increase in segmental wall thickening. From 19% ± 21% to 31% ± 30% at 5 mo (<i>P</i> < 0.001) and 47% ± 46% at 3 yr (<i>P</i> = 0.04)	Not mentioned	Not mentioned	Less ventricular remodeling in NMR at 3 yr

LVEF: Left ventricular ejection fraction; CTO: Chronic total occlusion; PCI: Percutaneous coronary intervention; NMR: Nuclear magnetic resonance.

myocardial ischemia should be revascularized in order to improve the prognosis must therefore be reviewed^[26]. In the context of patients with chronic coronary artery disease, the ISCHEMIA clinical trial will attempt to demonstrate whether the strategy of cardiac catheterization for revascularization is better than strategy of medical treatment in patients with moderate to severe ischemia detected in a stress test with imaging^[27]. In this trial, there will presumably be few patients with CTO, meaning that it is possible that its findings cannot be fully extrapolated to this specific population. As regards patients specifically with CTO, there are two ongoing clinical trials that are randomizing patients with angina or ischemia in an imaging test for medical treatment or angioplasty. The EURO-CTO clinical trial, being run at a European level, has the primary objective of evaluating quality of life at 12 mo, as well as assessing major coronary events after 3 years^[28]. The DECISION-CTO clinical trial, conducted in Asian countries, has a composite primary endpoint (cardiac death, myocardial infarction, stroke or further revascularization) evaluated after 3 years^[29].

CTO TREATMENT IN PATIENTS WITH VENTRICULAR DYSFUNCTION

Chronic hypoperfusion due to the presence of a CTO on a viable myocardium can cause ventricular dysfunction, and may lead to symptoms such as exercise intolerance and heart failure resulting from this dysfunction. It therefore seems logical that the opening of an occluded artery which irrigates a viable but dysfunctional myocardium could reverse this dysfunction and improve these

patients' symptoms and prognosis. There are few studies, all of which are observational, that have specifically addressed this issue (Table 4). Most available data suggest a very modest improvement in ventricular function as a result of opening an occluded artery. For example, Sirnes assessed the changes in ventricular function by ventriculography and was only able to demonstrate a 2% increase in ejection fraction, although the regional radial shortening increased by 16% in the revascularized areas^[30]. This slight improvement in regional ventricular function does not appear to depend on the presence of pre-existing collaterals, but probably on preserved microvascular integrity^[31]. The use of more accurate methods for quantifying ventricular function, such as cardiac magnetic resonance imaging, also confirms that the improvement in ventricular function as a result of opening an occluded artery is very modest^[32]. The improvement in the prognosis of patients with ventricular dysfunction due to revascularization is currently a topic of heated debate, following the results of randomized STICH study^[33]. In this clinical trial, patients with multivessel disease and ventricular dysfunction did not improve their prognosis as a result of revascularization surgery, in comparison with the medically treated group. Surprisingly, even the specifically studied patients with myocardial viability did not benefit from revascularization^[34]. The STICH study did not include patients with CTO, but the concept and the comments are relevant, because the percutaneous treatment of CTO is often justified simply on the basis of viability.

Meanwhile, the treatment of a CTO as a cause of deterioration in the ejection fraction due to complications

Table 5 Baseline characteristics of clinical and angiographic variables in studies included on Joyal meta-analysis^[42]

Ref.	Age (yr)		Male sex (%)		Multivessel disease (%)		Diabetes (%)		LVEF (%)		NYHA class 3-4 (%)		Renal dysfunction (%)		Occlusion length (mm)		Calcified vessel (%)		Ischemic burden	
	Success	Failure	Success	Failure	Success	Failure	Success	Failure	Success	Failure	Success	Failure	Success	Failure	Success	Failure	Success	Failure	Success	Failure
Finci <i>et al.</i> ^[42] , 1990	55 ± 11	55 ± 12	93	88	24	23	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Warren <i>et al.</i> ^[43] , 1990	54	55	53	47	48	52	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Ivanhoe <i>et al.</i> ^[44] , 1992	55 ± 10	56 ± 11	81	82	30	54 (0.0001)	10	15	55 ± 10	56 ± 11	3	3	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Angiot ^[45] , 2000	55 ± 10	56 ± 11	52	88	37	45	10	11	59 ± 14	59 ± 14	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Noguchi <i>et al.</i> ^[46] , 2000	61 ± 9	61 ± 11	78	80	47	67 (0.01)	26	32	56 ± 12	54 ± 9	n/d	n/d	n/d	11.3 ± 8.3	14.1 ± 8.1	37	56 (< 0.01)	n/d	n/d	n/d
Suero <i>et al.</i> ^[47] , 2001	60 ± 11	61 ± 12	78	80	73	82 (0.001)	21	20	51 ± 14	52 ± 14	n/d	n/d	8.2	7.1	n/d	n/d	n/d	n/d	n/d	n/d
Olivari <i>et al.</i> ^[48] , 2003	58 ± 10	59 ± 11	86	85	45	60 (0.014)	17	20	56 ± 10	56 ± 10	9	7	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Hoye <i>et al.</i> ^[49] , 2005	60 ± 11	61 ± 10	74	72	54	67 (0.03)	12	9.1	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Drozdz <i>et al.</i> ^[50] , 2006	57 ± 10	58 ± 10	81	80	46	53	11	11	n/d	n/d	14.4	18 (NYHA ≥ 2)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Aziz <i>et al.</i> ^[51] , 2007	59	59	76	81	50	40 (0.006)	14	9	53	53	12.2	15.7	0.3	1.8	n/d	n/d	n/d	n/d	n/d	n/d
Prasad <i>et al.</i> ^[52] , 2007	63 ± 11	64 ± 11	76	75	70	74	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Valenti <i>et al.</i> ^[53] , 2008	67 ± 11	70 ± 11	81	83	85	87	24	21	42 ± 13	41 ± 14	n/d	n/d	n/d	25 (15-52.5)	28 (21-47.5)	n/d	n/d	n/d	n/d	n/d
de Labriolle <i>et al.</i> ^[54] , 2008	61 ± 12	64 ± 10	72	87	45	66 (0.002)	19	40.5 (0.005)	50 ± 12	48 ± 15	n/d	n/d	9.1	6.3	n/d	n/d	n/d	n/d	n/d	n/d

LVEF: Left ventricular ejection fraction; n/d: No data; NYHA: New York Heart Association.

during the procedure should not be ruled out. In recent years, major breakthroughs have been described in the material used for the percutaneous treatment of CTO, which has led to a significant reduction in complications^[35]. However, the statement that today's complication rates are similar to those occurring in the treatment of less complex lesions could not be further from the truth. The Multinational CTO Registry mentions a rate of residual coronary dissection and perforation of 4.3% and 1.7% in successfully patients treated. However, among patients treated without success, these rates are 9.4% and 7.4%, respectively^[35]. In the series from a large Japanese centre, the overall rate of perforation in all percutaneous coronary intervention procedures is 1.2%, but 44% of these occur in patients with CTO^[36]. In another large Japanese centre, the rates of coronary dissection, perforation, distal embolization are 14.7%, 8.2% and 3.7% respectively, when an antegrade approach is used, and 10.1%, 13% and 1.4% respectively, when the procedure is performed *via* the retrograde route^[37]. Some authors have postulated that this high rate of complications in unsuccessfully treated patients partially explains their worse prognosis compared with those who are successfully treated^[38,39].

CTO TREATMENT TO IMPROVE PROGNOSIS

Some registries have reported that patients with complete revascularization have a better prognosis than those with incomplete revascularization, including the presence of

an untreated CTO^[40]. On this basis, the main argument which normally supports the treatment of CTO is that successfully treated patients have a better prognosis than those treated without success. This is apparent in the Joyal meta-analysis, in which successful treatment was associated with a significant improvement in mortality compared to unsuccessfully treated patients^[41]. This meta-analysis, conducted on studies with mainly retrospective data, seems to suggest that the baseline characteristics of successfully treated patients are similar to those treated without success, and that the unsuccessfully treated patients act as a medically treated control group. However, the studies performed with retrospective data^[42-54] often lack information on some of the baseline characteristics of patients which have a clear effect on prognosis (Table 5). When studies with prospectively collected data are analyzed, it becomes apparent that the baseline characteristics of patients treated without success are clearly different from those who are successfully treated. For example, the Canadian prospective registry contains many variables of poor prognosis among the unsuccessfully treated patients, such as having a longer history of prior infarction, prior multivessel disease, a longer CTO, higher rate of residual dissection and perforation during the procedure, which undoubtedly influences the these patients' poor prognosis^[36]. Furthermore, when the collection of variables is prospective and they are included in the predictive statistical model^[55], the benefit of successful treatment of CTO is cancelled out as these patients have baseline characteristics with a better prognosis than those treated without success. This hypothesis is corroborated by the recent publication of the long-term results of patients in the CREDO-Kyoto Registry^[5]. In this large series, the clinical evolution of 1192 successfully treated patients was compared with 332 unsuccessfully treated patients. Hospital mortality tended to be lower among the successfully treated patients than among those who were unsuccessfully treated (1.4% *vs* 3%, $P = 0.053$). During a three-year follow-up period, all-cause mortality did not differ between the two groups (9% *vs* 13.1%, $P = 0.18$), while the incidence of cardiac-related death was significantly lower in the successfully treated group (4.5% *vs* 8.4%, $P = 0.03$). However, after adjustment for confounding variables, successful treatment was not associated with either reduced total mortality (hazard ratio 0.93, 95%CI: 0.64 to 1.37, $P = 0.69$) or cardiac mortality (HR = 0.71, 95%CI: 0.44-1.16, $P = 0.16$). The only benefit associated with success in the treatment of CTO was a lower rate of surgical revascularization.

One group of patients deserves special consideration. These are patients with acute coronary syndrome in which the culprit artery is treated initially, but who have another chronically occluded artery which is considered for recanalization in a second procedure. This argument is based on the fact that these patients have a worse prognosis than patients with acute coronary syndrome with no CTO^[56]. The EXPLORE clinical trial, which randomizes patients with CTO with no culprit artery after an acute coronary artery syndrome on revas-

cularization treatment within 7 d of the ischemic event *vs* medical treatment^[57] attempts to clarify this important issue, which is currently performed frequently without any scientific evidence.

CONCLUSION

Treatment of CTO has emerged in recent years as a result of a revolution in medical equipment that enables these patients to be managed with success rates well above those of a few years ago. However, there is an urgent need for randomized studies to support this therapy as it is not risk-free, and is very expensive. Pending randomized studies, patients should be selected very carefully, especially if they are asymptomatic or very few symptoms, and the benefits obtained in terms of complications during the procedure, the quality of life obtained and further ischemic events avoided should be evaluated systematically.

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WJC 6th Anniversary Special Issues (5): Myocardial infarction

Significance of lead aVR in acute coronary syndrome

Akira Tamura

Akira Tamura, Department of Cardiology and Clinical Examination, Oita University, Yufu 879-5593, Japan

Author contributions: Tamura A wrote this topic highlight.

Correspondence to: Akira Tamura, Associated Professor, Department of Cardiology and Clinical Examination, Oita University, Idaigaoka -1, Hasama-machi, Yufu 879-5593, Japan. akira@oita-u.ac.jp

Telephone: +81-97-5865804 Fax: +81-97-5494245

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Core tip: In this article, I will review current evidence on lead aVR in the field of acute coronary syndrome.

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Abstract

The 12-lead electrocardiogram (ECG) is a crucial tool in the diagnosis and risk stratification of acute coronary syndrome (ACS). Unlike other 11 leads, lead aVR has been long neglected until recent years. However, recent investigations have shown that an analysis of ST-segment shift in lead aVR provides useful information on the coronary angiographic anatomy and risk stratification in ACS. ST-segment elevation in lead aVR can be caused by (1) transmural ischemia in the basal part of the interventricular septum caused by impaired coronary blood flow of the first major branch originating from the left anterior descending coronary artery; (2) transmural ischemia in the right ventricular outflow tract caused by impaired coronary blood flow of the large conal branch originating from the right coronary artery; and (3) reciprocal changes opposite to ischemic or non-ischemic ST-segment depression in the lateral limb and precordial leads. On the other hand, ST-segment depression in lead aVR can be caused by transmural ischemia in the inferolateral and apical regions. It has been recently shown that an analysis of T wave in lead aVR also provides useful prognostic information in the general population and patients with prior myocardial infarction. Cardiologists should pay more attention to the tracing of lead aVR when interpreting the 12-lead ECG in clinical practice.

INTRODUCTION

Lead aVR, an augmented and unipolar limb lead, was constructed to obtain specific information from the right upper portion of the heart, including the outflow tract of the right ventricle and the basal portion of the interventricular septum. However, lead aVR has been long neglected until recent years. This is thought to be because most cardiologists have considered that the tracing of lead aVR merely reflects reciprocal information from the lateral limb and precordial leads^[1]. However, in the last decade, evidence indicating the importance of lead aVR in the field of acute coronary syndrome (ACS) has been accumulating. In this article, the author will review current evidence on lead aVR in the field of ACS.

ST-SEGMENT SHIFT IN LEAD AVR

Prediction of acute left main trunk occlusion

Because the left coronary artery mostly supplies approximately 75% of the left ventricular (LV) myocardial mass, acute occlusion of the left main trunk (LMT) causes life-threatening hemodynamic deterioration and malignant arrhythmias, resulting in an adverse outcome. Therefore, a rapid diagnosis and subsequent urgent revascularization with percutaneous coronary intervention (PCI) or

coronary bypass surgery is very important in acute LMT occlusion. The 12-lead electrocardiogram (ECG) is a crucial tool in the diagnosis of ACS. Yamaji *et al*^[2] compared electrocardiographic findings among 16 patients with acute LMT occlusion, 46 patients with acute left anterior descending coronary artery (LAD) occlusion, and 24 patients with acute right coronary artery (RCA) occlusion and found that ST-segment elevation > 0.05 mV in lead aVR was more common in acute LMT occlusion (88%) compared to acute LAD occlusion (43%) and acute RCA occlusion (8%). Furthermore, the magnitude of ST-segment elevation in lead aVR greater than or equal to that of ST-segment elevation in lead V₁ was found to have 81% sensitivity and 80% specificity for differentiating acute LMT occlusion from acute LAD occlusion. They considered that ST-segment in lead aVR observed in acute LMT occlusion is caused by transmural ischemia in the basal part of the interventricular septum through impaired coronary blood flow of the first major septal branch arising from the LAD and that smaller ST-segment elevation in lead V₁ is due to the counterbalance of injury currents produced by transmural ischemia in both the anterior and posterior walls. The Yamaji's criterion requires validation by further studies with a large sample size.

In acute LMT occlusion, ST-segment elevation in lead aVR can also occur as a mirror image of ST-segment depression in the lateral limb and precordial leads. For example, global subendomyocardial ischemia caused by acute LMT occlusion can produce widespread ST-segment depression, especially in the lateral precordial leads, resulting in ST-segment elevation in lead aVR. In a review article, Nikus *et al*^[3] classify the electrocardiographic findings of acute LMT occlusion into the following patterns: (1) widespread ST-segment depression with maximal changes in lead V₄₋₆ with inverted T waves; (2) ST-segment elevation in lead aVR; and (3) anterior (anterolateral) ST-segment elevation. Ischemia-induced conduction disturbances, including right bundle branch block, left anterior fascicular block, and intraventricular conduction disturbance, are also frequently observed in acute LMT occlusion^[3]. The lack of one single uniform electrocardiographic pattern of acute LMT occlusion is thought to be greatly due to the heterogeneity of the amount and localization of the ischemic jeopardized myocardium.

In summary, the electrocardiographic findings of acute LMT occlusion do not show one single uniform electrocardiographic pattern. The classification proposed by Nikus and Eskola requires validation. Whether there is a specific electrocardiographic finding to predict a poor outcome in patients with acute LMT occlusion needs to be investigated.

ST-segment elevation in lead aVR in non-ST-segment-elevation ACS

Several studies have examined the significance of ST-segment elevation in lead aVR on the admission ECG in non ST-segment elevation ACS (NSTEMI-ACS)^[4-8]. Barrabés *et al*^[4] examined the association between ST-segment

shift in lead aVR and in-hospital mortality in 775 patients with a first non ST-segment elevation myocardial infarction (NSTEMI) and found that the rates of in-hospital mortality were 1.3% in 525 patients without ST-segment elevation in lead aVR, 8.6% in 116 patients with 0.05 mV to 0.1 mV of ST-segment elevation in lead aVR, and 19.4% in 134 patients with ST-segment elevation ≥ 0.1 mV in lead aVR. After adjusting for clinical variables, the odds ratios (ORs) for in-hospital mortality in the last 2 groups were 4.2 (95%CI: 1.5-12.2) and 6.6 (95%CI: 2.5-17.6), respectively. In 437 patients who underwent coronary arteriography within 6 mo of the onset of symptoms, the prevalence of LMT or 3-vessel disease among the 3 groups was 22.0%, 42.6%, and 66.3%, respectively. They concluded that in NSTEMI, ST-segment elevation in lead aVR is independently associated with increased in-hospital mortality probably because of severe coronary artery disease. In a GRACE substudy, including 5064 patients with NSTEMI-ACS, Yan *et al*^[5] showed that neither minor (0.05-0.1 mV) nor major (> 0.1 mV) ST-segment elevation in lead aVR was an independent predictor of in-hospital and 6-mo mortality after adjusting for other validated prognosticators in the GRACE risk model. The results are inconsistent with those of Barrabés *et al*^[4]. In the study of Yan *et al*^[5], the prevalence of ST-segment elevation > 0.1 mV in lead aVR was only 1.5% ($n = 76$), which was much lower compared to the study by Barrabés *et al*^[4]. A small number of patients with ST-segment elevation > 0.1 mV in lead aVR might have led to the negative result. In addition, entering ST-segment deviation in other leads and ST-segment elevation in lead aVR simultaneously into the multivariate analysis might have led to the negative result because all patients with ST-segment elevation > 0.1 mV in lead aVR had ST-segment deviation in other leads. Taglieri *et al*^[6] showed that ST-segment depression ≥ 0.05 mV in any lead plus ST-segment elevation ≥ 0.1 mV in lead aVR was independently associated with culprit LMT disease and increased in-hospital and 1-year cardiovascular deaths in 1042 patients with NSTEMI-ACS. In these three studies^[4-6], coronary arteriography was not performed in all patients.

There are a few studies^[7-9] to examine the significance of ST-segment elevation in lead aVR in NSTEMI-ACS patients undergoing emergent coronary arteriography. Kosuge *et al*^[7] analyzed ECGs of 310 patients with NSTEMI-ACS undergoing coronary arteriography and found that ST-segment elevation ≥ 0.05 mV in lead aVR was the strongest predictor of LMT or 3-vessel disease, with 78% sensitivity and 86% specificity. In another study, Kosuge *et al*^[8] examined the prognostic value of ST-segment elevation ≥ 0.05 mV in lead aVR in 333 patients with NSTEMI-ACS undergoing coronary arteriography and showed that ST-segment elevation ≥ 0.05 mV in lead aVR as well as serum troponin T level ≥ 0.1 ng/mL were independent predictors of 90-d adverse outcomes, including death, myocardial infarction (MI), or urgent revascularization. When the patients were divided into 4 groups based on ST-segment shift in lead aVR and serum troponin T levels, patients with ST-segment elevation ≥ 0.05 mV in lead aVR combined with

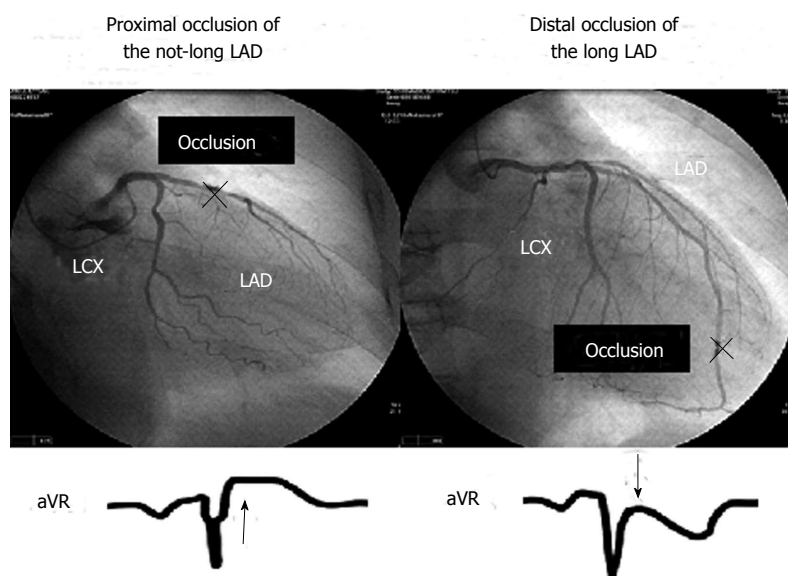


Figure 1 Association between ST-segment shift in aVR and coronary angiographic anatomy in a first anterior wall ST-segment elevation myocardial infarction. LAD: Left anterior descending coronary artery; LCX: left circumflex coronary artery.

an increased serum troponin T level had the highest rates of LMT or 3-vessel disease (62%) and 90-d adverse outcomes (47%). In another study, Kosuge *et al*^[9] examined 572 patients with NSTEMI-ACS undergoing coronary arteriography and showed that ST-segment elevation ≥ 0.1 mV in lead aVR identified severe LMT or 3-vessel disease ($\geq 75\%$ stenosis of LMT and/or 3-vessel disease with $\geq 90\%$ stenosis in ≥ 2 proximal lesions of the LAD and other major epicardial arteries), with 80% sensitivity and 93% specificity.

The current evidence suggests that in patients with NSTEMI-ACS, ST-segment elevation in lead aVR is associated with LMT or 3-vessel disease and increased adverse events. Considering the location of lead aVR, global subendomyocardial ischemia can produce ST-segment elevation in lead aVR. Therefore, it is reasonable that ST-segment elevation in lead aVR is associated with LMT or 3-vessel disease in NSTEMI-ACS.

ST-segment shift in lead aVR in anterior wall STEMI caused by LAD occlusion

A few studies^[10-12] have examined the significance of ST-segment shift in lead aVR on the admission ECG in first anterior wall STEMI caused by LAD occlusion. Kosuge *et al*^[10] analyzed ECGs of 105 patients with a first anterior wall STEMI undergoing successful reperfusion and found that 35 patients with ST-segment depression ≥ 0.05 mV in lead aVR had a larger infarct size, as estimated by peak creatine kinase levels, and a lower LV ejection fraction at predischage compared to 23 patients with ST-segment elevation ≥ 0.05 mV in lead aVR and 47 patients without ST-segment deviation in lead aVR. They speculated that ST-segment depression in lead aVR may reflect transmural ischemia extending to the apical and inferolateral walls, thereby resulting in a large MI. However, they did not evaluate the precise coronary angiographic anatomy. Accordingly, we^[11] examined the association between ST-segment shift in lead aVR and emergent coronary angiographic anatomy in 261 patients

with a first anterior wall STEMI and found that ST-segment depression ≥ 0.05 mV in lead aVR was associated with distal LAD occlusion (defined as occlusion of the LAD distal to the origin of the first septal branch) and a long LAD (defined as an LAD perfusing $\geq 25\%$ of the inferior wall) and that ST-segment elevation ≥ 0.05 mV in lead aVR was associated with proximal LAD occlusion (defined as occlusion of the LAD proximal to the origin of the first septal branch) and a not-long LAD. Interestingly, patients with proximal occlusion of the long LAD, who would suffer from a large MI, had a relatively lesser degree of ST-segment shift in lead aVR. We considered that this is due to the counterbalance of injury currents produced by transmural ischemia in both the basal part of the interventricular septum and the inferolateral and apical walls. In another study^[12], we examined the association between ST-segment shift in lead aVR and left ventriculography findings at predischage in 237 patients with a first anterior wall STEMI and found that LV ejection fraction at predischage did not differ significantly among 85 patients with ST-segment elevation ≥ 0.05 mV in lead aVR, 106 patients without ST-segment deviation, and 46 patients with ST-segment depression ≥ 0.05 mV in lead aVR. We concluded that both ST-segment elevation and depression in lead aVR may not be associated with a large infarct size in first anterior wall STEMI.

On the basis of the results of our 2 studies^[11,12], the association between ST-segment shift in lead aVR and emergent coronary angiographic anatomy in first anterior wall STEMI can be summarized as follows (Figure 1): (1) ST-segment elevation in lead aVR is more common in proximal occlusion of the not-long LAD; and (2) ST-segment depression in lead aVR is more common in distal occlusion of the long LAD. Acute LAD occlusion proximal to the origin of the first septal branch can produce ST-segment elevation in lead aVR through transmural ischemia in the basal portion of the interventricular septum, and acute occlusion of the long LAD can produce ST-segment depression in lead aVR though transmural

ischemia in the inferolateral and apical regions. However, it should be noted that the following conditions that can cause ST-segment elevation in lead aVR may disturb the theory: concomitant ischemia in the non-LAD region caused by multivessel disease, LV hypertrophy with strain pattern, and some types of conduction disturbances.

The current evidence suggests that in anterior wall STEMI caused by LAD occlusion, the length of the LAD and the site of occlusion of the LAD can affect ST-segment in lead aVR. The prognostic significance of ST-segment shift in lead aVR in such STEMI needs to be clarified.

Infarct-related coronary artery and ST-segment depression in lead aVR in inferior wall STEMI

Inferior wall STEMI can be caused by RCA or left circumflex coronary artery (LCX) occlusion, although the RCA is much more likely to be the infarct-related vessel. A few studies^[13-15] have examined whether ST-segment shift in lead aVR on the admission ECG can differentiate inferior wall STEMI caused by LCX occlusion from that caused by RCA occlusion. Nair *et al*^[13] analyzed admission ECGs in 30 patients with inferior wall STEMI and found that ST-segment depression ≥ 0.1 mV in lead aVR had 80% sensitivity and 96% specificity to identify LCX occlusion. Sun *et al*^[14] analyzed admission ECGs of 90 patients with inferior wall STEMI and showed that ST-segment depression ≥ 0.1 mV in lead aVR had 70.0% sensitivity and 94.3% specificity to identify LCX occlusion. In contrast, Kanei *et al*^[15] showed that ST-segment depression ≥ 0.1 mV in lead aVR had a high specificity (86%) but a low sensitivity (53%) to identify LCX occlusion in 106 patients with inferior wall STEMI. Thus, the diagnostic value of ST-segment depression in lead aVR to identify LCX occlusion in inferior wall STEMI is not yet established.

The current evidence suggests that in inferior wall STEMI, ST-segment depression in lead aVR is more common in LCX occlusion than in RCA occlusion. Large population studies are needed to determine the diagnostic value of ST-segment depression in lead aVR to identify LCX occlusion in inferior wall STEMI.

Significance of ST-segment depression in lead aVR in inferior wall STEMI

A few studies^[15-17] have examined the association between ST-segment depression in lead aVR on the admission ECG and infarct size in inferior wall STEMI. Menown *et al*^[16] examined 173 patients with ST-segment elevation ≥ 0.1 mV in inferior or lateral (I, aVL, V₅, and V₆) leads and found that ST-segment elevation ≥ 0.1 mV in inverted lead aVR (lead -aVR) was associated with a larger infarct size, as estimated by peak creatine kinase levels. Kosuge *et al*^[17] examined 225 patients with a first inferior wall STEMI and found that the degree of ST-segment depression in lead aVR was an independent predictor of impaired myocardial reperfusion defined as myocardial blush grade of 0 or 1. They considered that in inferior wall STEMI, ST-segment depression in lead aVR reflects transmural ischemia extending to the inferolateral and apical walls

and that it therefore relates to a larger infarct size and impaired myocardial reperfusion. Kanei *et al*^[15] reported that ST-segment depression ≥ 0.1 mV in lead aVR was associated with a large infarct size, as estimated by peak creatine kinase levels, in 86 patients with inferior wall STEMI caused by RCA occlusion but not in 19 patients with inferior wall STEMI caused by LCX occlusion. In 86 patients with RCA occlusion, the prevalence of a large posterolateral branch was higher in 12 patients with ST-segment depression ≥ 0.1 mV in lead aVR than in 74 patients without it (67% *vs* 16%, $P = 0.0006$). They considered that acute occlusion of the RCA with a large posterolateral branch occlusion can cause transmural ischemia extending to the inferolateral and apical walls, resulting in ST-segment depression in lead aVR and that it therefore relates to a larger infarct size. Since their study included only 19 patients with LCX occlusion, the association between ST-segment depression in lead aVR and infarct size in inferior wall STEMI caused by LCX occlusion needs to be further investigated.

The current evidence suggests that in inferior wall STEMI caused by RCA occlusion, ST-segment depression in lead aVR is associated with the RCA with a large posterolateral branch, which would result in a large MI. The prognostic significance of ST-segment depression in lead aVR in inferior wall STEMI needs to be determined by further studies with a large sample size.

Large population studies on the prognostic significance of ST-segment shift in lead aVR in STEMI

There are two large-population studies^[18,19] to examine the prognostic significance of ST-segment shift in lead aVR on the admission ECG in STEMI. In a HERO-2 substudy, including 15315 patients with STEMI, Wong *et al*^[18] found a U-shaped relationship between ST-segment shift in lead aVR and 30-d mortality in anterior wall STEMI. In inferior wall STEMI, only ST-segment elevation ≥ 0.1 mV in lead aVR was independently associated with increased 30-d mortality. However, the underlying mechanisms for the observations are unclear, because that study did not evaluate the coronary angiographic anatomy. In an APEX-AMI substudy^[19], including 5683 patients with STEMI treated by PCI, ST-segment elevation ≥ 0.1 mV in lead aVR was independently associated with increased 90-d mortality (HR = 5.87, 95%CI: 2.09-16.5) in inferior wall STEMI, whereas ST-segment depression ≥ 0.1 mV in lead aVR was independently associated with increased 90-d mortality (HR = 1.53, 95%CI: 1.06-2.22) in non-inferior wall STEMI. However, the results have to be interpreted with some cautions. First, the precise mechanisms responsible for the observations are unclear, because that study did not evaluate the detailed coronary angiographic anatomy (the site of occlusion and the length of the coronary arteries). Second, both the inferior wall STEMI group and the non-inferior wall STEMI group included patients with STEMI caused by LMT, LAD, RCA, LCX, or graft occlusion, among whom the outcome would be different. Therefore, the heterogeneity of each group might have affected the results.

The current evidence suggests that the prognostic significance of ST-segment shift in lead aVR may differ according to the site of STEMI. The exact prognostic significance of ST-segment shift in lead aVR in anterior wall STEMI and inferior wall STEMI remains to be determined.

T-WAVE ABNORMALITY IN LEAD AVR

Although numerous studies have examined the association between T-wave abnormalities with or without ST-segment changes and cardiovascular events, the significance of T-wave abnormality in lead aVR has not been investigated until recent years. Tan *et al*^[20] firstly examined the association between T-wave amplitude in lead aVR and cardiovascular mortality during a mean follow-up period of 4 years in 24270 male veterans whose electrocardiograms were obtained for any clinical reasons. In that study, an upright (> 0 mV) T wave in lead aVR was found to be associated with increased cardiovascular mortality after adjusting for age and heart rate (HR = 2.8, 95%CI: 2.3-3.3). Anttila *et al*^[21] examined the prognostic impact of a positive T wave (≥ 0 mV) in lead aVR in 6254 subjects aged ≥ 30 years who participated in the field healthy examination. In that study, the positive T wave in lead aVR was observed in 2.2% of the subjects, and the relative risk for cardiovascular mortality for the positive T wave in lead aVR was 2.94 (95%CI: 1.47-2.49) after adjusting for other risk factors. In a NHANES sub-study, including 7928 participants aged > 40 years, Badheka *et al*^[22] showed that a positive (> 0 mV) T wave in lead aVR was the strongest multivariate predictor of cardiovascular mortality (OR = 3.37, 95%CI: 2.11-5.36) and that the addition of T-wave amplitude in lead aVR to the Framingham risk score improved model discrimination and calibration with better reclassification of intermediate-risk subjects. However, in these three studies^[20-22], the underlying mechanisms for these observations are not identified.

A few studies^[23,24] have investigated the significance of T-wave positivity in lead aVR in prior MI. We^[23] examined 122 patients with anterior wall prior MI and found that 20 patients with a T wave (≥ 0.1 mV) in lead aVR had higher pulmonary arterial, pulmonary capillary wedge, and LV end-diastolic pressures, a lower cardiac index, and a lower LV ejection fraction than 102 patients without such a T wave in lead aVR. The prevalence of a long LAD was significantly higher in the former group than in the latter group (60% *vs* 30.4%, $P = 0.01$), and none of the former group had an LAD that did not reach the apex. We concluded that in anterior wall prior MI, the positive T wave in lead aVR is associated with severely reduced cardiac function, with the long LAD. In another study, we^[24] examined the prognostic significance of an upright (> 0 mV) T wave in lead aVR in 167 patients with a prior MI and found that the upright T wave in lead aVR was independently associated with increased cardiac death or hospitalization for heart failure for a follow-up period of 6.5 ± 2.8 years (HR = 3.10, 95%CI: 1.23-7.82).

However, because of a relatively small sample size, we could not evaluate the prognostic significance of the upright T wave in lead aVR in each of anterior wall MI and non-anterior wall MI.

The current evidence suggests that the positive T wave in lead aVR is associated with cardiovascular mortality in the general population and patients with prior MI. Further studies are needed to clarify the underlying mechanisms for increased cardiovascular mortality in subjects with a positive T wave in lead aVR in the general population and determine the prognostic significance of the positive T wave in lead aVR in anterior wall MI and non-anterior MI.

Q WAVE IN LEAD -AVR

In normal subjects, QRS configuration in lead aVR indicates QS pattern. We have noticed that a Q wave in lead -aVR (R wave in lead aVR) is sometimes observed in patients with anterior wall MI. Accordingly, we examined the association between a prominent Q wave (duration ≥ 20 ms) in lead -aVR and LV wall motion at predischARGE in 87 patients with a first anterior wall STEMI^[25]. In that study, 17 patients with a prominent Q wave in lead -aVR on the predischARGE ECG was found to have a lower LV ejection fraction and more reduced regional wall motion in the apical and inferior regions than 70 patients without a Q wave in lead -aVR. Furthermore, the former had a higher prevalence of a long LAD compared to the latter (70.6% *vs* 32.9%, $P = 0.01$), and none of the former group had an LAD that did not reach the apex. We concluded that in anterior wall STEMI, the prominent Q wave in lead -aVR is associated with severe regional wall motion abnormality in the apical and inferior regions, with the long LAD. Further studies are needed to clarify the clinical and prognostic significance of the prominent Q wave in lead -aVR in anterior wall MI and non-anterior wall MI.

ORDERLY DISPLAY OF THE LIMB LEADS

The conventional display of the 6 precordial leads provides an anatomically contiguous view of the electrical activity progressing horizontally from the right anterior (V_1) to left lateral (V_6). In contrast, the conventional display of the 6 limb leads provides only a suboptimal representation of the electrical activity on the frontal plane. The 6 limb leads are anatomically better to be displayed by the following sequence: aVL, I, -aVR, II, aVF, and III (Figure 2). This orderly display of the 6 limb leads (known as the Cabrera format or sequence) provides a 150° view of the heart at regular 30° intervals. When using the orderly display, we can globally visualize the electrical activity on the frontal plane and easily understand the localization of the transmurally ischemic myocardium on the frontal plane in the setting of STEMI. The orderly display of the 6 limb leads has been routinely used in Sweden since the late 1970s. In 2009, the AHA/ACCF/HRS recommends that ECG machines should be equipped with switching systems that will allow the limb leads to be displayed and

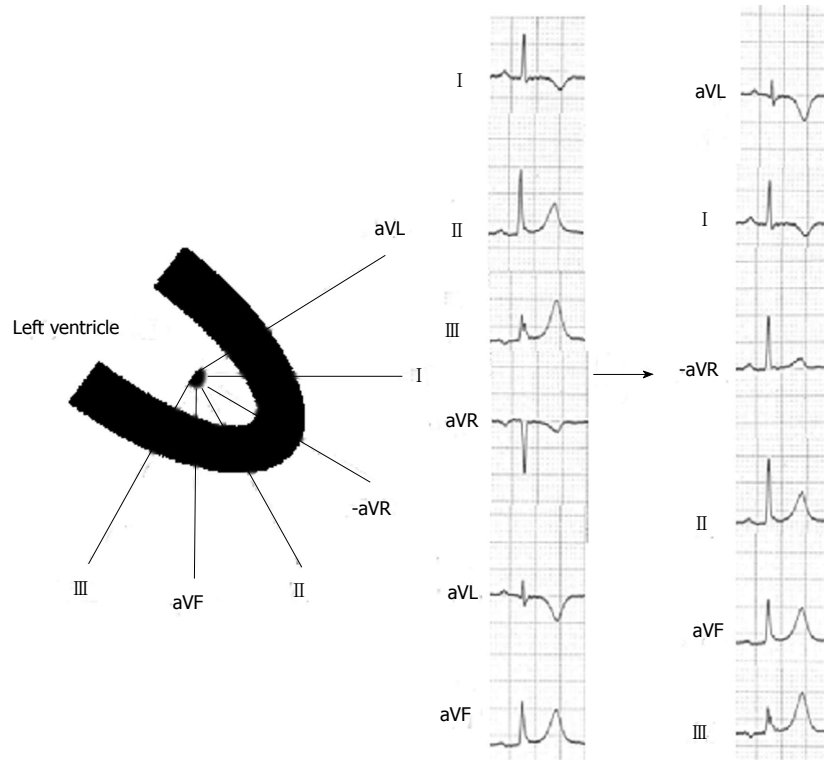


Figure 2 Orderly display of the 6 limb leads.

Table 1 Possible mechanisms of ST-segment elevation or depression in lead aVR and coronary angiographic anatomy in acute coronary syndrome

Lead aVR	Possible mechanisms
ST-segment elevation	Global subendomyocardial ischemia caused by LMT or 3-vessel disease Transmural ischemia in the basal portion of the interventricular septum caused by proximal LAD (especially, not-long LAD) occlusion Transmural ischemia in the right ventricular outflow tract caused by proximal occlusion of the RCA with a large conal artery Reciprocal changes opposite to ischemic or non-ischemic ST-segment depression in the lateral limb and precordial leads
ST-segment depression	Transmural ischemia in the inferolateral and apical regions caused by occlusion of the long LAD (especially, distal occlusion) Transmural ischemia in the inferolateral and apical regions caused by occlusion of the RCA with a large posterolateral branch Transmural ischemia in the inferolateral and apical regions caused by occlusion of the LCX (especially, with impaired coronary blood flow of the obtuse marginal or posterolateral branch that perfuses the inferolateral and apical regions)

LMT: Left main trunk; LAD: Left anterior descending coronary artery; RCA: Right coronary artery; LCX: Left circumflex coronary artery.

Table 2 Current evidence concerning the prognostic significance of ST-segment elevation or depression in lead aVR in acute coronary syndrome

Type of ACS	Findings of previous studies
NSTE-ACS	ST-segment elevation in lead aVR was independently associated with increased in-hospital mortality ^[4] Neither minor (0.05-0.1 mV) nor major (> 0.1 mV) ST-segment elevation in lead aVR was an independent predictor of in-hospital or 6-mo mortality ^[5] ST-segment depression ≥ 0.05 mV in any lead plus ST-segment elevation ≥ 0.1 mV in lead aVR was independently associated with increased in-hospital and 1-year cardiovascular deaths ^[6] ST-segment elevation ≥ 0.05 mV in lead aVR was an independent predictor of 90-d adverse outcomes, including death, myocardial infarction, or urgent revascularization ^[8]
Anterior wall STEMI	U-shaped relationship between ST-segment shift in lead aVR and 30-d mortality was observed ^[18]
Non-inferior wall STEMI	ST-segment depression ≥ 0.1 mV in lead aVR was independently associated with increased 90-d mortality ^[19]
Inferior wall STEMI	ST-segment elevation ≥ 0.1 mV in lead aVR was independently associated with increased 30-d mortality ^[18] ST-segment elevation ≥ 0.1 mV in lead aVR was independently associated with increased 90-d mortality ^[19]

ACS: Acute coronary syndrome; NSTE: Non ST-segment elevation; STEMI: ST-segment elevation myocardial infarction.

labeled appropriately in their anatomically contiguous sequences^[26]. This useful display of the 6 limb leads should

be routinely used in everyday clinical practice.

CAUTIONS WHEN INTERPRETING PREVIOUS DATA ON LEAD AVR

It should be noted that the point at which the magnitude of ST-segment elevation or depression in lead aVR was measured varies among previous studies on ST-segment shift in lead aVR. In “Third universal definition of MI”^[27], abnormal ST-segment elevation or depression measured at the J point is defined. Therefore, the clinical and prognostic significance of ST-segment shift in lead aVR measured at the J point has to be determined in various conditions of ACS.

CONCLUSION

Accumulating evidence indicates that the analysis of ST-segment shift in lead aVR provides useful information on the coronary angiographic anatomy and risk stratification in various conditions of ACS. The possible mechanisms of ST-segment elevation or depression in lead aVR in ACS and the current evidence concerning the prognostic significance of ST-segment elevation or depression in lead aVR in ACS are summarized in Tables 1 and 2, respectively. It has been also shown that the analysis of T wave in lead aVR provides useful prognostic information in the general population and patients with prior MI. Cardiologists should pay more attention to ST-segment shift and T-wave positivity in lead aVR in everyday clinical practice.

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Calpain system and its involvement in myocardial ischemia and reperfusion injury

Christiane Neuhof, Heinz Neuhof

Christiane Neuhof, Heinz Neuhof, Department of Internal Medicine I, Cardiology, Justus-Liebig-University of Giessen, 35392 Giessen, Germany

Author contributions: All authors contributed equally to this review.

Correspondence to: Christiane Neuhof, MD, Department of Internal Medicine I, Cardiology, Justus-Liebig-University of Giessen, Klinikstrasse 33, 35392 Giessen, Germany. christiane.neuhof@innere.med.uni-giessen.de

Telephone: +49-641-98556690 Fax: +49-641-9919876

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Abstract

Calpains are ubiquitous non-lysosomal Ca^{2+} -dependent cysteine proteases also present in myocardial cytosol and mitochondria. Numerous experimental studies reveal an essential role of the calpain system in myocardial injury during ischemia, reperfusion and postischemic structural remodelling. The increasing Ca^{2+} -content and Ca^{2+} -overload in myocardial cytosol and mitochondria during ischemia and reperfusion causes an activation of calpains. Upon activation they are able to injure the contractile apparatus and impair the energy production by cleaving structural and functional proteins of myocytes and mitochondria. Besides their causal involvement in acute myocardial dysfunction they are also involved in structural remodelling after myocardial infarction by the generation and release of proapoptotic factors from mitochondria. Calpain inhibition can prevent or attenuate myocardial injury during ischemia, reperfusion, and in later stages of myocardial infarction.

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Key words: Calpain; Calpain inhibition; Calcium overload; Myocardial injury; Ischemia; Reperfusion; Myocardial infarction; Remodelling

Core tip: Calpains, calcium-dependant cytosolic cysteine proteases, are essentially involved in the pathophysiology of myocardial infarction. Their inhibition has shown in animal experiments an enhanced tolerance towards ischemia, a reduction of myocardial infarction and reperfusion injury, and an improvement of the process of remodelling. The availability of specific calpain inhibitors offers new prophylactic and therapeutic possibilities for patients with myocardial infarction, revascularisation and coronary surgery.

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INTRODUCTION

Calpains are calcium-dependent, cytosolic cysteine proteases and are expressed as two “ubiquitous” isoenzymes (μ - and m -calpains) and several “tissue specific” isoforms (n -calpains). Their primary structure contains as well calmodulin-like calcium-binding proteins as well as papain protease-like components, reflected by the term calpain^[1]. A non-lysosomal Ca^{2+} -activated cysteine protease was isolated for the first time by Guroff^[2] 1964 from rat brain. Calpains are meanwhile found in all cells of vertebrates that have been examined^[2-5], in cells of invertebrates^[6,7] and fungi^[8], but not in bacteria and plants.

Besides their physiological functions they are also implicated in pathophysiological processes^[4,9-12], especially with disturbed calcium homeostasis^[4,13,14]. Thus, calpains were found to be involved in myocardial tissue damage resulting from ischemia and reperfusion^[15,16]. Calpain inhibition on the other hand ameliorates, respectively, prevents these lesions in animal experiments with potential prophylactic and therapeutic implications even in clinical

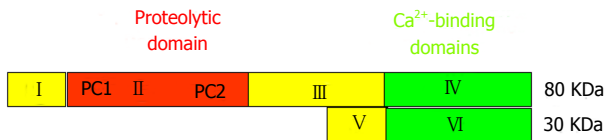


Figure 1 Domain structure of the catalytic 80-kDa and the regulatory 30-kDa subunits of the μ - and m-calpain dimers.

situations.

The following review will give an overview of the physiological and pathophysiological basis of the calpain system and finally focus on its role in myocardial ischemia, infarction and reperfusion and the effectiveness of calpain inhibition based on experimental studies.

BASICS OF THE CALPAIN SYSTEM

Nomenclature

The terms μ -calpain and m-calpain were first used by Cong *et al.*^[17] in 1989. They indicate the micromolar (μ -calpain) respectively millimolar (m-calpain) Ca^{2+} -concentrations required for their activation. Thus, μ -calpain is activated in the presence of 3-50 $\mu\text{mol/L}$ Ca^{2+} and m-calpain in the presence of 400-800 $\mu\text{mol/L}$ Ca^{2+} ^[17,18]. Meanwhile, more than 25 proteins with structural similarities were identified as calpains or calpain-like molecules. The genes assigned to 15 of these proteins are numerically named as CAPN1 up to CAPN15 and their coded molecules are named as calpain1 up to calpain15, correspondingly. Calpain1 as well as Calpain2 are biologically active as proteases not as monomers but only as dimers with an identical 30-kDa subunit each. Both biologically active calpains are usually called μ -calpain (calpain 1 + 30-kDa subunit) and m-calpain (calpain 2 + 30-kDa subunit), respectively^[4,12].

According to Suzuki *et al.*^[19] calpains are subdivided into two main categories: (1) "typical" calpains with a calmodulin-like domain IV at their COOH-terminus; and (2) "atypical" calpains without this component. Typical calpains are μ -calpain, m-calpain and the calpains 5, 7, 10, 13 and 15 which are also named "ubiquitous" calpains as they are present in almost all cells of vertebrates. In contrast to the "ubiquitous" calpains the "tissue-specific" calpains are exclusively expressed in special cells and tissues, such as calpain 3 in skeletal muscle^[20], calpain 6 in placenta and embryonic muscles^[21], calpain 8 and 9 in the gastrointestinal tract^[22], calpain 11 in the testis^[23], and calpain 12 in hair follicles^[24].

Domain structure of μ - and m-calpain

Both proteases μ - and m-calpain exist as dimers with two subunits of 80-kDa and 30-kDa each (Figure 1)^[25,26]. The larger 80-kDa catalytic subunits of μ -calpain and m-calpain are coded in humans by different genes on chromosome 11 respectively chromosome 1^[27]. On the base of their amino acid sequences they are composed of four regions/domains: (1) a N-terminal domain; (2) a catalytic CysPc protease domain consisting of two protease core regions PC1 and PC2; and (3) a C2-like Ca^{2+} -regulated

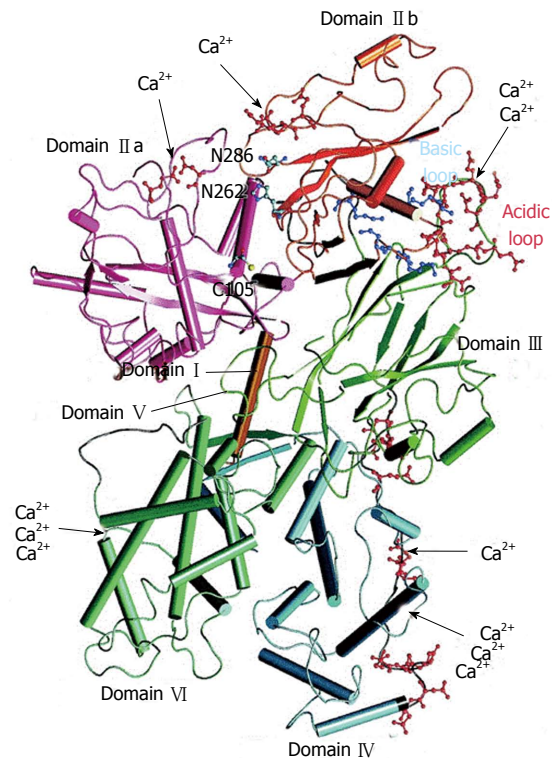


Figure 2 Crystallographic structure of human m-calpain by Suzuki *et al.*^[33].

phospholipid-binding domain, and IV a Ca^{2+} -binding penta-EF-hand domain^[28-31].

Domain I contains an amphipathic alpha-helix in the N-terminus of μ -calpain which was shown to be important in targeting and migrating of μ -calpain into the intermembrane space of mitochondria. Domain I of m-calpain, however, does not contain a similar N-terminal component^[32].

Domain II represents the catalytic CysPc protease domain. It consists of two separate protease core domains PC1 with a cysteine (Cys) residue and PC2 with a histidine (His) residue and an asparagine (Asn) residue. These residues form a catalytic triade as known from cysteine proteases such as papain or cathepsin (Figure 2). Both core domains PC1 and PC2 have Ca^{2+} -binding sites for a single Ca^{2+} by each^[33,34].

Domain III is structurally related to C2 domains and can bind phospholipids in a Ca^{2+} -dependent manner. It links the Ca^{2+} -binding domains with the catalytic domain II and is supposed to be involved in the adjustment of the calpain activity *via* electrostatic interactions^[35].

Domain IV shows a slight sequence homology to calmodulin (51%-54%) and has five Ca^{2+} -binding COOH-terminal EF-hand motifs. The fifth motif binds to the corresponding EF-hand sequences of domain VI of the smaller 30 kDa subunit and, thus, contributes to the dimer formation of both calpain subunits^[4,31,33,36].

The smaller regulatory 30 kDa subunit, responsible for the stability of the larger catalytic subunit, consists of the N-terminal Gly-rich domain V and the Ca^{2+} -binding calmodulin-like penta-EF-hand domain VI. The long stretches of Gly residues and an unordered structure of

Calpastatin is the only known specific endogenous inhibitor and regulator of μ - and m-calpain. In addition also H-kininogen and $\alpha 2$ -macroglobulin are inhibiting calpain besides other proteases^[57]. Human calpastatin is encoded by a single gene on chromosome 5^[58] and expressed in several isoforms from 17.5 to 107 kDa^[59-61].

It consists of four inhibitory domains I, II, III and IV, and one N-terminal domain L without inhibitory capability^[62, 63]. Each inhibitory unit inhibits one calpain molecule competitively by blocking the substrate access to the catalytic centre^[64, 65]. Calpastatin inhibits exclusively calpain and not other proteases^[57]. Binding of calpastatin to calpain and its inhibition is Ca^{2+} -dependent. The Ca^{2+} -concentrations for this are lower as needed for the half-maximal proteolytic activity of μ - and m-calpain^[66]. Calpains and calpastatin are found in physical proximity within the cells^[67, 68]. Therefore, mechanisms are necessary to enable calpain to perform its biological purpose, since calpastatin already binds to calpain with increasing Ca^{2+} -concentrations. Thus, the translocation of calpain to the membranes could cause a spatial distance to calpastatin. Furthermore, special mechanisms/factors could lower the threshold for Ca^{2+} to activate calpain without influencing the binding of calpastatin^[3]. With regard to activation and deactivation of calpain many questions are still open concerning a regulating, respectively, modifying role of substrate phosphorylation.

Localization of μ - and m-calpain in cell and tissue

In all examined cells of vertebrates μ -calpain, m-calpain and calpastatin are found at least as the only constituents of the calpain system or they exist in various combinations with great varying patterns of distribution. Thus, human erythrocytes and platelets only contain μ -calpain, and smooth muscles of vessels and stomach predominantly contain m-calpain, whereas, in skeletal muscles and kidneys of the most representatives of vertebrates nearly equal amounts of μ - and m-calpain are found^[67, 69, 70]. Both calpains as well as calpastatin are exclusively localized intracellular and apparently associated with subcellular structures. Thus, 93% of the μ -calpain are found in human red blood cells within the cytosol and 7% membrane associated^[71]. Most of the μ -calpain, m-calpain and calpastatin is localized close to the Z-disc in the myofibrils of skeletal and cardiac muscle, smaller amounts are found in the I- and A-bands. In mitochondria and nuclei only a tenth, respectively, a fifth of calpains and calpastatin was identified compared to their concentration in the Z-disc region^[67, 72, 73]. Calpain and calpastatin are normally localized with a close spatial proximity.

Substrates for calpain

Normally, calpains have only access to intracellular substrates, whereby their cleavage decisively depends on the local activity of calpain and its inhibitor calpastatin. Many proteins are cleaved by calpains *in vitro*, but there is no conclusive evidence that they cannot also be splitted by calpain *in vivo*.

Calpain cleaves the cytoskeleton and membrane-associated proteins: adducin^[74], ankyrin^[75], caldesmon^[9], cadherin^[76, 77], C-protein^[78], desmin^[79], dystrophin^[80], the filamin/actin-binding proteins MAP1 and MAP2^[81], myosin^[82], the neurofilament-proteins NFH, NFM and NFL^[83], NR2-subunit^[84], the anchoring protein PSD-95

of NMDA-receptors^[85], α II-spectrin^[16], β -spectrin^[86], talin^[87, 88], titin^[89], tropomyosin and troponin I^[78], troponin T^[90], vimentin^[79, 91], and vinculin^[92].

Furthermore, kinases, phosphatases and transcription factors are cleaved, such as: EGF-receptor-kinase^[93], myosin light-chain kinase^[94], protein-kinase C^[95], calcineurin^[96], inositol-polyphosphat-4-phosphatase^[97], protein-tyrosin-phosphatase-1B^[98], the transcription factors c-Jun, c-Fos^[99, 100], and p53^[101, 102].

PHYSIOLOGICAL FUNCTIONS AND PATHOPHYSIOLOGICAL IMPLICATIONS OF THE CALPAIN SYSTEM

Physiological function of μ - and m-calpain

Calpains are not seen to play an essential role in the intracellular protein digestion. In contrast to lysosomal proteases and the proteasome calpains split proteins by a limited proteolysis into large fragments with potential regulatory and signalling functions^[4]. Many studies including experiments with transgenic mice indicate, that calpains are involved in the embryonic development and cell function^[103-105], cytoskeletal/membrane attachments/cell motility^[79, 81, 86-88, 106], intracellular signal transduction^[95, 107-109], cell cycle^[110, 111], regulation of gene expression^[99, 101], apoptosis^[112-115], and in the long-term potentiation of synaptic transmission^[84, 85, 116].

Involvement of calpains in inherited and acquired diseases

A lacking synthesis of calpains or the dysregulation of the calpain activity disturbing the proteolysis of structural and regulatory proteins is found in a series of genetic and acquired diseases, such as: limb girdle muscular dystrophy (LGMD2A)^[117, 118], muscular dystrophy (type Duchenne and Becker)^[119], diabetes mellitus (type 2)^[120], gastric cancer^[121], Alzheimer's disease^[122-125], multiple sclerosis^[126, 127], and cataract formation^[127].

THE KEY ROLE OF CALCIUM HOMEOSTASIS WITHIN THE CALPAIN SYSTEM

Regulation of Ca^{2+} -homeostasis

Many vital cell functions are regulated by the concentration of intracellular available Ca^{2+} , such as muscle contraction, neurotransmitter release, glandular secretion, and intercellular communication^[128, 129]. And last but not least, calpains are Ca^{2+} -activated proteases. Because of its key role, normally the Ca^{2+} concentration is controlled at different cellular levels *via* mitochondria, plasmalemma/sarcolemma and endoplasmatic reticulum. The transmembrane transport of ions is regulated actively, selectively and directionally-oriented by voltage gated ion channels, by ATP-consuming ion pumps (Na^+ - K^+ -ATPases, Ca^{2+} -ATPases, proton-ATPases) and by the concentration gradient due to carrier proteins (Na^+ / H^+ -exchanger,

$\text{Na}^+/\text{HCO}_3^-$ -symporter, $\text{Na}^+/\text{Ca}^{2+}$ -exchanger)^[130-133]. Failing of this control mechanisms may result in an excessive intracellular accumulation of Ca^{2+} (Ca^{2+} -overload) with severe cellular dysfunction up to cell death^[14,134,135].

Events with increasing myocardial Ca^{2+} concentration

Studies with isolated perfused mammalian hearts have shown an increasing cytosolic Ca^{2+} concentration during hypoxia in hearts of rabbits^[136] and ferrets^[137], during ischemia in hearts of rabbits^[138] and rats^[139], and during post-ischemic reperfusion in hearts of rats^[140] and ferrets^[141]. Severe burn trauma also augments the Ca^{2+} content in myocytes^[142,143] and mitochondria^[144] of rat hearts. The same effect can be observed upon exposure of isolated perfused rabbit hearts^[145] and isolated rat cardiomyocytes^[146,147] to hydroxyl free radicals. In analogy to the heart, a Ca^{2+} -overload was also observed in rat brains^[148,149] during hypoxia/ischemia and in the spinal cord^[150] after traumatization.

Disturbance of Ca^{2+} homeostasis in the heart:

Pathomechanisms and consequences

The underlying mechanisms and consequences of an imbalance in Ca^{2+} homeostasis are documented the most extensively in heart during hypoxia, ischemia and post-ischemic reperfusion. They are initiated by the decreasing ATP generation and developing acidosis resulting from oxygen deficiency. The activation of the Na^+/H^+ -exchanger (NHE-1)^[152,151,152], which causes the influx of Na^+ into the cell for exchange with H^+ in order to regulate pH, and the simultaneous inhibition of the Na^+/K^+ -ATPase^[153], due to lack of ATP, plays a key role in the intracellular Ca^{2+} -overload. Thus, Na^+ accumulates intracellular and lowers the transmembranous Na^+ gradient, which is the driving force behind the $\text{Na}^+/\text{Ca}^{2+}$ -exchanger by transporting Ca^{2+} out off the cell, resulting in Ca^{2+} -accumulation. The $\text{Na}^+/\text{Ca}^{2+}$ -exchanger which represents a bidirectional transport system is also able to transport Ca^{2+} in exchange with Na^+ in a reverse mode into the cell^[152,154,155]. Driving forces for this are the increasing intracellular Na^+ concentration and depolarisation of the sarcolemma.

Today, disturbance of Ca^{2+} -homeostasis is seen as the main triggering factor of cardiac dysfunction and myocardial injury during ischemia and reperfusion, such as the myocardial stunning, a long-lasting reversible reduction of heart contraction after ischemia^[156-158], or like the Ca^{2+} -overload induced hypercontracture during reperfusion/reoxygenation^[14,159-161], or the incidence of arrhythmias during reperfusion^[162]. Other factors, such as reactive oxygen species or inflammation seem to play a minor role in these situations^[163].

Many studies demonstrate as a consequence of an increasing intracellular Ca^{2+} -concentration the activation of calpains, which cleave numerous functional and structural proteins, and thereby decisively contribute to ischemic and postischemic injury. Thus, the activation of the calpain system during hypoxia or ischemia is well documented in the myocardium of rats^[164-167] and humans^[168],

as well as in the brain of rats^[169-171]. In rat renal proximal tubules hypoxia induces the increase of μ -calpain activity^[172], whereas calpain inhibition reduces the renal functional and structural damage following ischemia and reperfusion^[173]. Hypoxia was also found to up-regulate the activity and gene expression of calpains in endothelial cells of the pulmonary artery^[174].

ROLE OF CALPAINS IN MYOCARDIAL ISCHEMIA/REPERFUSION INJURY

Global ischemia

Most studies on the implication of calpains for myocardial dysfunction and failure are based on experiments in isolated perfused mammalian hearts, in which the duration of perfusion stop (global ischemia) is restricted to enable at least a recovery with reperfusion.

Global ischemia in isolated perfused rat hearts was found to induce a time-dependent translocation of m-calpain to the membrane initially not associated with calpain activation which occurred only during reperfusion and intracellular pH normalization^[175]. Under comparable conditions, a loss of myofibrillar desmin, α -actinin, and spectrin was observed in guinea pig hearts, which was reduced by calpain inhibitor I^[176]. Immunohistochemical studies revealed the proteolysis of caldesmon and α -fodrin at the intercalated discs and the sarcolemma after post-ischemic reperfusion in rat hearts. Degradation of both proteins could be suppressed and myocardial function improved by calpain inhibitor I^[16,177]. The inhibition of α -fodrin degradation associated with the attenuation of myocardial dysfunction could also be observed after cardioplegic cardiac arrest in rat hearts in the presence of calpain inhibitor SNJ-1945^[178]. As a result of calpain activation, the essential Ca^{2+} -handling proteins Ca^{2+} -ATPase (SERCA2a) and the SERCA regulatory protein PLB were degraded upon global ischemia and reperfusion in a working rat heart preparation. Their degradation, the depression of cardiac performance and the release of lactate dehydrogenase, indicating the myocardial damage, could be significantly attenuated by calpain inhibition with calpain inhibitor III (MDL28170)^[179]. As an indicator of myocardial tissue damage creatine phosphokinase and lactate dehydrogenase are released from myocytes into the perfusion fluid during reperfusion in concentrations dependent on the duration of ischemia (Figure 4). Calpains seem to be responsible or to contribute to these effects, as calpain inhibition with A-705239 significantly reduces the enzyme release^[180].

Cardiac muscle contraction is initiated by Ca^{2+} via troponin/tropomyosin which are known as substrates of calpain. Therefore, their cleavage is supposed to be jointly responsible for myocardial dysfunction in ischemia/reperfusion injury. With regard to this, degradation of troponin T (TnT) was observed during ischemia/reperfusion of isolated perfused rat hearts and was reduced by calpain inhibition with PD150606 and PD151746^[181]. In addition, "overexpression of calpastatin by gene trans-

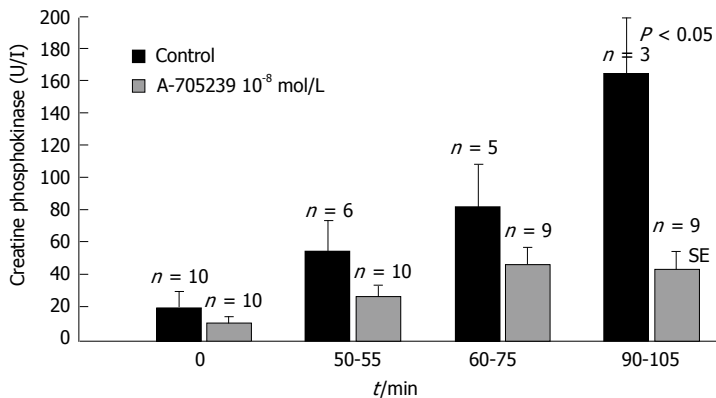


Figure 4 Release of creatine phosphokinase into the perfusion fluid of isolated rabbit hearts subjected to ischemia and reperfusion^[180]. Control experiments without inhibitor are represented by black-coloured columns and inhibitor (A-705239 10⁻⁸ mol/L) treated hearts by grey-coloured columns. Data are expressed as means \pm SE of $n = 10$ experiments each. Both groups differ significantly ($P < 0.05$) at the end of reperfusion.

fer prevents troponin I (TnI) degradation and ameliorates contractile dysfunction in rat hearts subjected to global ischemia followed by reperfusion^[182].

Mitochondrial function impairment

Damage of mitochondria plays a central role in the pathophysiology of reperfusion injury *via* the impairment of oxidative metabolism, respectively, energy production and the generation and accumulation of metabolic products toxic to the myocytes. Cardiac mitochondria are located subsarcolemmal beneath the plasma membrane and interfibrillar between the myofibrils^[183-185]. In animal and human hearts μ -calpain, m-calpain and calpain 10 are present in cytosol and in the intermembrane space of mitochondria^[67,186-189]. Cytosolic calcium content is found to increase in hearts of rats and rabbits during myocardial ischemia and reperfusion and is made responsible for the subsequent activation of calpains^[190,191]. The damage of Ca²⁺-handling proteins by direct cleaving or detaching the Na⁺/K⁺-ATPase and the Na⁺/Ca²⁺-exchanger from their binding ankyrin^[174,192], and by proteolysis of sarcoplasmic reticulum Ca²⁺-ATPase (SERCA)^[179,193] and Ryanodine receptor RyR^[194], sustains Ca²⁺-influx and calpain activation and aggravates myocardial injury. Thus, SERCA2a and the SERCA regulatory protein PLB were found to be degraded upon global ischemia and reperfusion in a working rat heart preparation. Their degradation, the depression of cardiac performance and the release of lactate dehydrogenase, indicating the myocardial damage, could be significantly attenuated by calpain inhibition with calpain inhibitor III (MDL28170)^[179].

One of the most serious consequences of mitochondrial damage by calpains is the impairment of oxidative phosphorylation with loss of ATP generation. Damage to mitochondrial oxidative metabolism can be caused on various levels of the electron transport chain (ETC). In isolated renal cortical mitochondria from rats and rabbits calpain 10 was shown to cleave complex I subunits of the ETC, which could be prevented by pretreatment with calpeptin^[195]. The impairment of mitochondrial respiration is documented in isolated perfused rabbit hearts^[180,196]. State 3 respiration decreased significantly during 45 min of global ischemia and further decreased during 60 min of reperfusion, and this reaction could be

significantly attenuated by addition of calpain inhibitor A-705239 to the perfusion fluid (Table 1).

Reduced state 3 respiration reflects the impairment of the electron transport chain (ETC), above all complex I, which is an early target of myocardial ischemia^[197].

Calpain inhibitor A-705239 administered before ischemia and reperfusion also attenuated the increase in permeability of the inner mitochondrial membrane (mitochondrial permeability transition), as reflected by the reduced state 4 respiration and leak-respiration^[180].

Besides their deleterious effect on mitochondrial oxidative metabolism, calpains are also recognized to cause the generation and release of substances toxic to myocytes.

During reperfusion, mitochondria generate reactive oxygen species that lead to additional mitochondrial and myocyte injury^[197-200].

Dependent on the degree of oxidative damage in concert with mitochondrial calcium overload and calpain activation, mitochondrial permeability transition can occur by formation of inner membrane pores^[201,202]. Mitochondrial permeability transition can result in disruption of the outer mitochondrial membrane and the release of cytochrome c, a key step inducing apoptosis^[203]. Cytochrome c is detectable in the cytosol of rabbit myocardium at 30 min of ischemia^[204], whereas cytochrome c content decreases in subsarcolemmal mitochondria^[205]. Mitochondrial calpain plays an important role in programmed cell death by generation or release of apoptotic factors in mitochondria during ischemia and reperfusion. Thus, the cleavage of Bid, a pro-apoptotic BH3-only Bcl-2 family member, is documented in isolated perfused adult rabbit hearts during ischemia/reperfusion, and in secondary *in vitro* studies recombinant Bid was cleaved by calpain to an active fragment that was able to mediate cytochrome c release^[206]. It was also shown, that activated mitochondrial μ -calpain, mostly located in the intermembrane space, cleaves and releases apoptosis inducing factor (AIF) from isolated mouse heart mitochondria. Besides, mitochondrial μ -calpain activity increased in buffer perfused mouse hearts during ischemia/reperfusion whereas the mitochondrial AIF content decreased. Inhibition of mitochondrial μ -calpain using MDL-28170 preserved the AIF content within the mitochondria and

Table 1 Effect of calpain inhibitor A-705239 on impairment of mitochondrial function following myocardial ischemia and reperfusion^[180]

	<i>n</i>	State 3 respiration (nmol O ₂ /min per milligram)	State 4 respiration (nmol O ₂ /min per milligram)	RCI (state3 rate): (state 4 rate)	Leak respiration(nmol O ₂ /min per milligram)	Stimulation by cytochrome c %
Control						
Before ischemia	4	6.4 ± 1.1	0.5 ± 0.1	12.5 ± 2.7	0.15 ± 0.07	6.0 ± 10.0
Ischemia 45 min	8	3.5 ± 1.4 ^{a,c}	0.9 ± 0.3 ^a	4.4 ± 2.5 ^a	0.32 ± 0.14 ^a	10.0 ± 6.0
Reperfusion 60 min	4	2.6 ± 1.3 ^{a,c}	0.9 ± 0.3 ^a	3.2 ± 2.1 ^a	0.43 ± 0.29	28.0 ± 16.0
A-705239 treated hearts						
Before ischemia	4	6.8 ± 1.3	0.6 ± 0.1	12.4 ± 1.1	0.12 ± 0.06	16.0 ± 9.0
Ischemia 45 min	9	5.0 ± 0.8 ^{a,c}	0.6 ± 0.2	8.2 ± 2.3 ^{a,c}	0.20 ± 0.14 ^a	15.0 ± 13.0
Reperfusion 60 min	5	4.2 ± 1.2 ^{a,c}	0.7 ± 0.2	6.4 ± 2.7 ^a	0.26 ± 0.24	

Data are presented as means of 4 to 9 experiments mean ± SD measured as duplicates or triplicates. A significant difference from baseline before ischemia is represented by ^a*P* < 0.05, and between both groups by ^c*P* < 0.05.

reduced cardiac injury^[186].

Partial ischemia and myocardial infarction

In contrast to models of global ischemia, in the experimental setting of partial ischemia by temporary occlusion of coronary arteries the duration of ischemia can be extended in time to enable irreversible myocardial damage to a restricted area with myocardial infarction without the risk of early global heart failure with reperfusion. In isolated perfused rat hearts it was shown, that during a 30 min occlusion of the left anterior descending coronary artery calpain translocates to the cell membranes without being activated initially. Calpain activation, as indicated by the hydrolysis of α -fodrin, only started with the onset of reperfusion and could be prevented by calpain inhibition with MDL-28170, just as the infarct size could be reduced by 32%^[175].

Inhibition of α -fodrin degradation and improvement of left ventricular function by calpain inhibitor SNJ-1945, administered 30 min before a gradual and partial coronary occlusion, was also found after mild ischemic-reperfusion in another study in rat hearts^[207]. Protecting effects of calpain inhibition on myocardial injury could also be demonstrated by own experiments with inhibitor administration both before and during reperfusion. "Two novel calpain inhibitors (A-705239 and A-705253) were studied in isolated perfused rabbit hearts subjected to a 60 min occlusion of the ramus interventricularis of the left coronary artery (below the origin of the first diagonal branch), followed by 120 min of reperfusion^[208,209]. The inhibitors were added to the perfusion fluid in various final concentrations from the beginning of the experiments before the coronary artery was blocked. The infarct size was significantly reduced in presence of both calpain inhibitors. The best effect was achieved with 10⁻⁸ mol/L A-705253 which reduced the infarcted area by 61.8 % (Figure 5A). In a second study in isolated perfused rabbit hearts subjected to a 60 min occlusion of the ramus interventricularis of the left coronary artery followed by 120 min of reperfusion calpain inhibitor A-705253 and/or the Na⁺/H⁺-exchange inhibitor cariporide[®] were added to the perfusion fluid at the beginning of reperfusion solely or in combination^[210]. The infarct size was signifi-

cantly reduced dose-dependently in presence of both inhibitors (Figure 5B). The best effect was achieved with 10⁻⁶ mol/L A-705253, which reduced the infarcted area by 33.6%. Cariporide[®] (10⁻⁶ mol/L) reduced the infarct size in the same extent. The combination of both inhibitors, however, didn't further improve cardioprotection. Thus, the protective effect can be attributed exclusively to its influence on the calpain system, since the combination of both inhibitors didn't augment the protective effect of sole calpain inhibition. The calpain inhibitor A-705253 is known to directly block the catalytic centre of activated calpains, whereas the Na⁺/H⁺-exchange inhibitor cariporide[®] prevents or reduces the ischemic intracellular Ca²⁺-overload and thus prevents or reduces the following calpain activation". This is shown in postischemic perfused rat and rabbit hearts where reduced calpain activation^[211] and calcium overload^[212] were observed upon inhibition of Na⁺/H⁺-exchange. Even in patients undergoing coronary bypass surgery pretreatment with cariporide[®] reduced mortality and the risk of myocardial infarction^[213], however, cerebrovascular events increased^[214]. In accordance with the findings in rabbit hearts, also in pigs undergoing occlusion of the left anterior descending coronary artery for 45 min followed by 6 h of reperfusion infarct size was reduced by 35% and hemodynamic alterations attenuated using calpain inhibitor A-705253^[215]. In experiments with isolated mouse hearts undergoing ischemia and reperfusion infarct size was decreased and ventricular function improved in calpain-1 knockout mice, whereas myocardial injury was greatly increased in transgenic mice hearts with calpain-1 overexpression^[216].

No sufficient information is available to what extent polymorphonuclear leukocytes (PMN) contribute to ischemic/reperfusion injury. In one study in isolated rat hearts perfused with PMNs, exposed to 20 min of ischemia and followed by 45 min of reperfusion, calpain inhibition with Z-Leu-Leu-CHO reduced the adherence of PMNs to the vascular endothelium and improved ventricular function, however, controls without PMNs are missing^[217]. Thus, with regard to the numerous experiments discussed in this review, which were all performed without PMNs in the perfusion fluid, polymorphonuclear leukocytes appear not to be essential for reperfusion

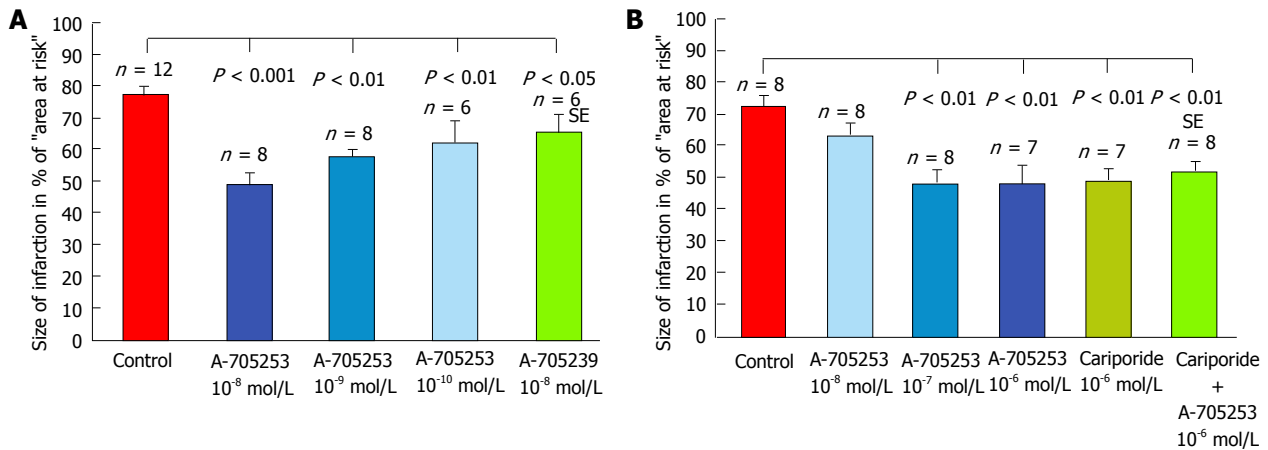


Figure 5 Development of myocardial infarction in isolated perfused rabbit hearts after occlusion of ramus interventricularis of left coronary artery for 60 min, followed by 120 min of reperfusion^[209]. A: The inhibitors were added to the perfusion fluid before ischemia; B: With reperfusion. Infarct size is expressed in percentage of the area at risk (the transiently not perfused myocardium). Control experiments without inhibitor are represented by a red-coloured column and inhibitor treated hearts by blue-coloured columns. Data are presented as means \pm SE. Infarct size is significantly reduced by calpain inhibition in all treated hearts compared to untreated controls.

injury.

Remodelling after myocardial infarction

Myocardial infarction is followed by a progressive structural remodelling of the heart, replacing and reconstructing the irreversibly damaged myocardium^[186,203,206]. After the early phase of ischemia-induced myocyte necrosis a longer lasting myocyte death by apoptosis can be observed. Proapoptotic factors are generated and released from myocardial mitochondria already during ischemia and reperfusion which are considered to be essentially involved in remodelling after myocardial infarction^[186,203,206]. Characteristics of apoptosis, DNA fragmentation and chromatin condensation, could be detected in isolated perfused rabbit hearts subjected to 30 min ischemia and 4 h reperfusion^[220]. In ischemic/reperfused rat hearts undergoing 30 min coronary occlusion followed by 6 h reperfusion the administration of calpain inhibitor I (CAI) 10 min before reperfusion significantly reduced DNA fragmentation and infarct size^[221]. Comparable results were achieved in mouse hearts with persistent coronary artery ligation for 4 d. Calpain inhibition with calpeptin was started 15 min before artery occlusion and continued during the observation time. Calpeptin administration reduced apoptotic cell death, as detected by TUNEL staining, and reduced infarct size and myocardial dysfunction^[222]. The important contribution of calpains to the process of myocardial remodelling is also documented by a transgenic mouse model with cardiomyocyte-specific deletion of gene *Capn4* (*Capn4-ko*) which is indispensable for μ - and m-calpain stability and activity. Mice were subjected to persistent left coronary artery ligation and followed up for 30 d. Deletion of *Capn4* reduced infarct expansion, apoptosis, myocardial remodelling and dysfunction^[223].

CONCLUSION

Numerous studies have shown an essential contribution

of calpains in myocardial injury following ischemia and reperfusion. Proven prevention or attenuation of post-ischemic heart damage by calpain inhibition with various tested inhibitors could offer a novel prophylactic or therapeutic approach for patients with myocardial infarction, revascularisation and coronary surgery.

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Neuregulin-1/erbB activities with focus on the susceptibility of the heart to anthracyclines

Cecilia Vasti, Cecilia M Hertig

Cecilia Vasti, Cecilia M Hertig, Laboratory of Molecular Cardiology, Institute for Research in Genetic Engineering and Molecular Biology - Dr. Hector N. Torres- (INGEBI), 1428 Buenos Aires, Argentina

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Correspondence to: Cecilia M Hertig, PhD, INGEBI, Laboratory of Molecular Cardiology, Institute for Research in Genetic Engineering and Molecular Biology - Dr. Hector N. Torres- (INGEBI), Vuelta de Obligado 2490, 1428 Buenos Aires, Argentina. chertig@dna.uba.ar

Telephone: +54-11-47832871 Fax: +54-11-47868578

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Abstract

Neuregulin-1 (NRG1) signaling through the tyrosine kinase receptors erbB2 and erbB4 is required for cardiac morphogenesis, and it plays an essential role in maintaining the myocardial architecture during adulthood. The tyrosine kinase receptor erbB2 was first linked to the amplification and overexpression of *erbB2* gene in a subtype of breast tumor cells, which is indicative of highly proliferative cells and likely a poor prognosis following conventional chemotherapy. The development of targeted therapies to block the survival of erbB2-positive cancer cells revealed that impaired NRG1 signaling through erbB2/erbB4 heterodimers combined with anthracycline chemotherapy may lead to dilated cardiomyopathy in a subpopulation of treated patients. The ventricular-specific deletion of either *erbB2* or *erbB4* manifested dilated cardiomyopathy, which is aggravated by the administration of doxorubicin. Based on the exacerbated toxicity displayed by the combined treatment, it is expected that the relevant pathways would be affected in a synergistic manner. This review examines the NRG1 activities that were monitored in

different model systems, focusing on the emerging pathways and molecular targets, which may aid in understanding the acquired dilated cardiomyopathy that occurs under the conditions of NRG1-deficient signaling.

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Key words: Ventricular dilation; Cardiotoxicity; ErbB2; ErbB4; Neuregulin; Trastuzumab; Doxorubicin

Core tip: We have reviewed the cardiac requirement of neuregulin-1 (NRG1) signaling through the receptor tyrosine kinase erbB2/erbB4. The evidence indicates that the NRG1/erbB signaling pathway displays a panel of activities implicated in maintaining the myocardial architecture during remodeling, which may explain why the combined treatment with antibodies against erbB2 and anthracycline chemotherapy may evolve into a severe dilated cardiomyopathy. We have further examined the potential molecular targets, which have been either inferred from impaired NRG1 signaling or directly assessed by the administration of NRG1. The current working hypotheses have been delineated towards a prospective molecular understanding of NRG1 signaling in heart.

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INTRODUCTION

Dilated cardiomyopathy (DCM) results from the abnormal remodeling of the myocardium with the eccentric growth of cardiomyocytes in response to valve defects, toxic and metabolic causes or gene defects^[1,2]. An ac-

quired form of DCM is manifested in a subpopulation of breast cancer patients treated with anthracycline chemotherapy combined with humanized antibodies against erbB2. The amplification and over-expression of erbB2 occurs in 25% of all breast cancer types, inducing a highly invasive tumor that has a poor prognosis when treated using conventional therapies. Targeted therapies with antibodies against erbB2 were shown to be clinically effective for erbB2-positive breast cancer patients through an objective tumor regression analysis, with lower rates of both recurrence and mortality^[3]. However, the iatrogenic effect of the combined immunotherapy and chemotherapy results in increased incidence of dilated cardiomyopathy, initially affecting a subpopulation of 27% of treated patients^[4].

We reviewed the panel of activities of the NRG1 pathway that affect cardiomyocyte survival, proliferation, differentiation and specification to further focus on the synergistically deregulated molecular pathways under the experimental conditions of impaired NRG1 signaling and doxorubicin therapy.

THERAPEUTIC CONSIDERATIONS

Evidence from long-term retrospective analyses of treatment with the humanized antibodies against erbB2 (trastuzumab) have suggested that deficient NRG1 signaling sensitizes the heart to anthracycline cardiotoxicity^[5,6]. These studies prompted the sequential administration of immunotherapy after chemotherapy in patients with no signs of cardiotoxicity to reduce the incidence of cardiomyopathy. The continuous development of immunotherapeutic drugs aimed at improving efficacy in tumor cell death have recently provided a novel humanized monoclonal antibody against erbB called pertuzumab, which prevents erbB receptor dimerization and thus blocks the activity of both erbB2 and erbB4. Currently, there are ongoing clinical trials about the safety and efficacy of these immunotherapeutics at escalating doses, when used to treat a diverse group of patients with epithelial-derived cancers. Thus far, the results from the CLEOPATRA study group indicate the beneficial effect of the combined action of pertuzumab and trastuzumab with docetaxel, which leads to the significantly progression-free and prolonged survival of patients with breast cancer while having a comparable level of cardiotoxicity both to previous formulations and to placebo with trastuzumab and docetaxel^[7]. These results prompted the FDA priority review of pertuzumab for its approval and release into the market in June 2012.

In the light of the cardiac adverse effects of the therapeutics used to block the survival of cancer cells, researchers have indicated that drugs, specifically those that target signaling pathways or kinase receptors and have a broad range of effects on cancer cells, should also be studied for cardiac safety^[8]. In this regard, the multidisciplinary workshop of the Association of the European Society of Cardiology aimed for a consensus in the management of treatments while prioritizing the awareness

of anthracycline cardiotoxicity and the development of new targeted therapeutics^[9].

Interestingly, the design of an observational trial on cardiotoxicity in cancer therapies has included an additional evaluation of cardiac risk incidence by analyzing the Single Nucleotide Polymorphism/haplotype variations in the NRG1/ErbB signaling gene components (NCT01173341). In addition to its impact on disease management, the results from this trial may contribute to the knowledge of genetic modifiers by identifying polymorphisms on the genes of the NRG1/erbB pathway that are associated with disease.

THE COMPONENTS OF THE NRG1 PATHWAY

Neuregulins are transmembrane proteins of four isotypes (NRG1-4). Neuregulin-1 is classified into at least three subgroups (type I - III) and has approximately 30 isoforms as a result of its synthesis from different promoters and splicing variants^[10]. Neuregulins: of types I and II are processed at the membrane by metalloproteinase, ADAM17, 19 and are cleaved by α -secretase activity^[11]. The release rate of the amino-terminal active domain is modulated by protein kinase C (PKC)-delta^[12]. The active peptide of the NRG is related to the epidermal growth factor (EGF), which contains a cysteine-rich domain that binds to and activates the tyrosine kinase receptors erbB4 and erbB3, which belong to the EGF receptor (erbB1) family. The active forms of erbB2 and erbB3 receptors are considered heterodimers because they lack either an opened ligand binding domain or tyrosine kinase activity, respectively, as opposed to the potential function of erbB4 receptor homodimers^[13].

In the heart, the active domain of NRG1 secreted from endothelial cells binds and activates the erbB2/erbB4 heterodimers expressed in cardiomyocytes (Figure 1). The NRG1 pathway, which was initially characterized as inducing cardiomyocyte differentiation and specification, has been identified as inducing a broader panel of activities according to the experimental model system and the induced heart condition (Table 1).

THE NRG1 INTRACELLULAR SIGNALING CASCADE

The erbB-dependent intracellular cascades have been extensively studied because of their important role in cancer cells, thereby providing a basis for analyses of signaling mechanisms in other cell types. The NRG activation of erbB receptors mediates the auto- and trans-phosphorylation of tyrosine residues at the receptor intracellular domain. A subgroup of phosphotyrosine residues bind specific adaptor molecules (*e.g.*, Grb, Shc, Src, SH3 domain)^[14], ultimately inducing intracellular pathways, *e.g.*, MAP kinase and PI 3'-kinase cascades, PLC γ , the regulation of the Ca^{2+} -dependent PKC and Nuclear Factor of Activated T cells activity (Figure 2)^[13,15].

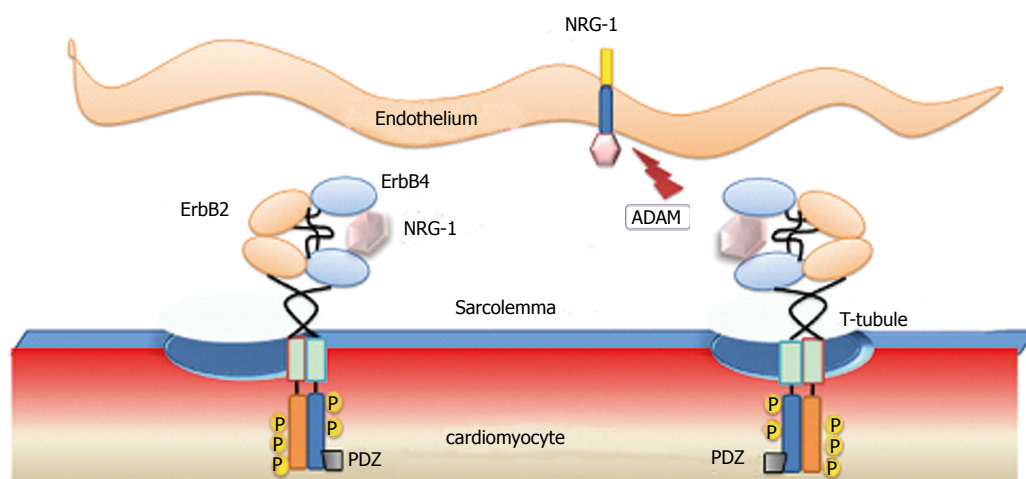


Figure 1 Endothelium-Cardiac muscle interactions through paracrine neuregulin-1 signaling. Secreted neuregulin-1 from endothelial cells binds to erbB4 inducing auto- and trans-phosphorylation of ErbB2/ErbB4 heterodimers, expressed in cardiomyocytes. NRG-1: Neuregulin-1; PDZ: Postsynaptic density 95, disc large and zona occludens-1 homologous protein domain; ADAM: A disintegrin and metalloproteinase; P: Phosphorylated tyrosine.

Table 1 Biological effects of neuregulin-1/erbB in embryonic and postnatal cardiomyocytes

Experimental system	Monitored response	Intracellular signal	Ref.
Cultured cells			
NRG1 administration in neonatal cardiomyocytes	Sarcomeric F-actin polymerization	PI 3'-kinase	[16]
	Myofibrillogenesis - Growth	Ras-Mek-erk1/2	[17]
	Proliferation	PI 3'-kinase	[17]
	NOS activation		[42]
	Muscarinic activation		[43]
	Karyo- cytokinesis		[44]
NRG1 administration in human ESC	Specification of cardiomyocytes of the conduction lineage		[45]
Animal models			
NRG1b injection to <i>ex vivo</i> developing E12.5 dpc mouse	Differentiation of trabeculae		[17]
	DNA synthesis, Proliferation	PI 3'-kinase	[17]
NRG1 administration in mouse with heart failure	Improved cardiac performance		[40]
Hypomorphic NRG1 deficiency in E8.5dpc mouse	Destabilization of gene regulatory network in the left ventricle	Erk1/2	[39]
Mouse ventricular-erbB2-KO	Overt dilation and reduced survival in erbB2 F/- postnatal mouse		[31]
Mouse ventricular-erbB2-KO	Dilation with cellular apoptosis		[32]
Mouse ventricular-erbB4-KO	Overt ventricular dilation at 3 mo and reduced survival		[33]
Lapatinib inhibition of erbB1/erbB2 in Mouse	erbB2-physiologic hypertrophy in pregnancy	MEK1/erk1/2	[34]
Adult ventricular-erbB4-KO	Cell division in myocardial infarction		[44]

Summary of cardiac activities of neuregulin-1 (NRG1)-erbB2/erbB4 signaling inferred from *in vitro* system and animal model studies. The NRG1 activities, -proliferation, myofibrillogenesis, ventricular remodeling, and repair-, were assessed based on the outcomes of the exogenous administration of NRG1 or by the complete or partial loss of erbB2/erbB4 signaling. NOS: Nitric oxide synthase; MEK1: Mitogen activated kinase erk kinase 1; ESC: Embryonic stem cells.

A link between NRG1 and focal adhesion kinase (FAK) has been observed in proliferative and migrating cells.

The Ras/MAPK/erk1/2 pathway was required for the NRG1-driven myofibrillogenesis in cultured cardiomyocytes. This activity was mimicked by a constitutively active form of Ras and inhibited by both its dominant negative form and the MEK1 inhibitor PD98059^[16,17]. The NRG1-induced ability of cardiomyocytes to proliferate was manifested by its combined administration with Insulin-like growth factor I (IGF-I). The NRG1/IGF-I induction of cardiomyocyte DNA synthesis in both *ex vivo* embryonic development and cultured neonatal cardiomyocytes was prevented by wortmannin, an inhibitor of PI3'-Kinase. Cellular transfection with an adenovirus harboring a constitutively active Akt mimicked the proliferative and protective activities, which were inhibited

by a dominant negative form of Akt in the presence of NRG1/IGF-I (Figure 2)^[17].

Alternative pathways may be activated by the cross-communication of erbB2 and G protein coupled receptors. Pro-hypertrophic GPCR agonists (*e.g.*, angiotensin II, endothelin I and isoproterenol) have been implicated in the transactivation of EGFR and erbB2, thereby inducing hypertrophic and survival stimuli in cardiac cells^[18,19].

ErbB non-phosphorylated interactions

Intracellular signaling also depends on the binding ability of specific non-phosphorylated residues of erbB receptors to PDZ (postsynaptic density 95, discs large and Zonula occludens-1) domain-containing proteins. This interaction with PDZ domain proteins is relevant for the

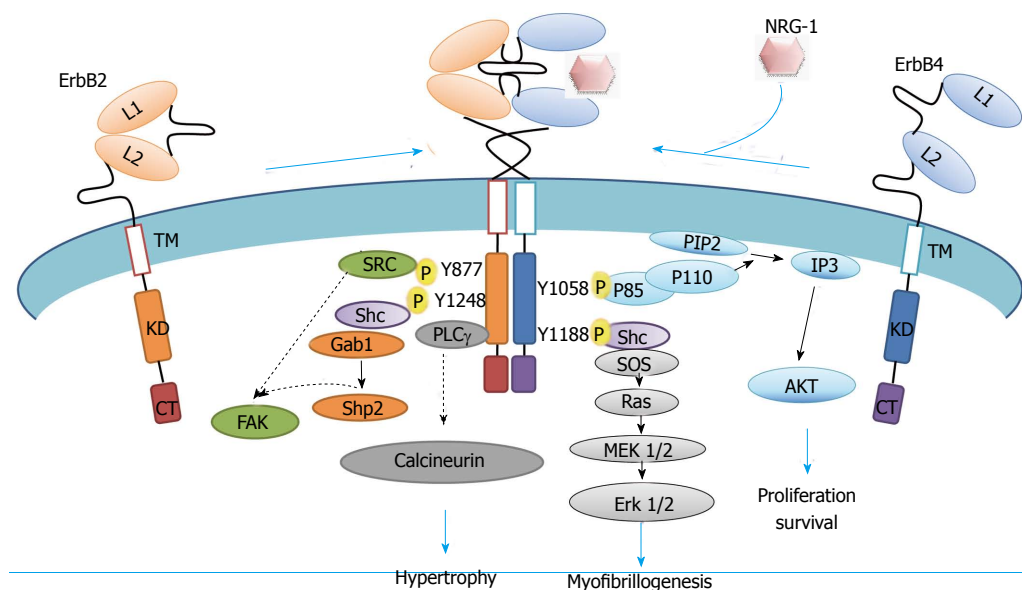


Figure 2 Representation of neuregulin-1-erbB2/erbB4 intracellular signaling cascade. Schematic representation of active ErbB2/ErbB4 heterodimers through phosphorylation, which phosphosites are docking sites for intracellular molecules involved in pathways that modulate myocyte biology. Specific non-phosphorylated residues interact to PDZ domain proteins. CT: Cytoplasmic tail; KD: Kinase domain; L: Ligand binding site; TM: Transmembrane domain; NRG-1: Neuregulin-1; PIP2: Phosphoinositol-2-phosphate; SOS: Son of sevenless; IP3: Inositol triphosphate; AKT: Thymoma viral oncogene homolog 1, a serine/threonine protein kinase; MEK: Mitogen activated kinase erk kinase; Shc: Src homology domain containing transforming protein; Shp: Protein tyrosine phosphatase; FAK: Focal adhesion kinase; Gab: Binding protein of growth factor bound protein Grb2; P: Phosphorylated tyrosine residues.

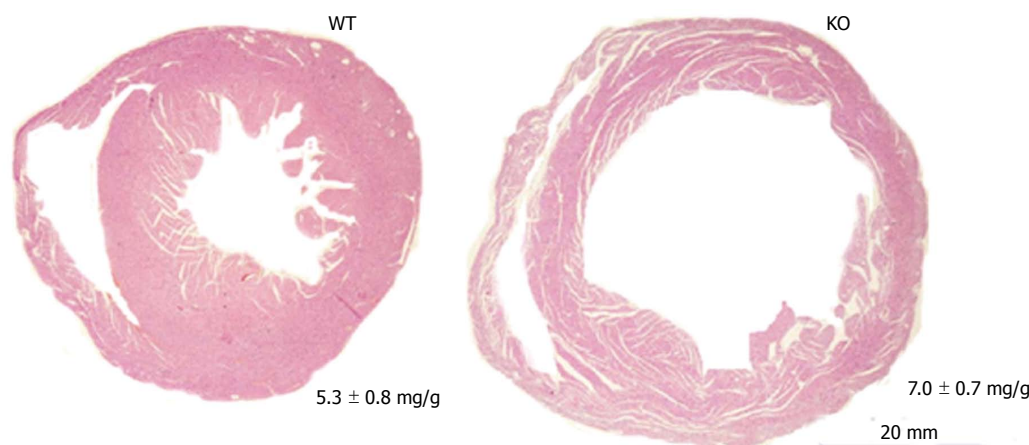


Figure 3 Ventricular specific erbB4-knockout leads to adult dilated cardiomyopathy. Representative image of transverse ventricular sections stained with hematoxylin-eosin. Camber dilation is overt in mouse erbB4-KO hearts in the adulthood. WT: Wild type; KO: Knock-out.

specific location of erbB proteins in particular membrane compartments and for the modulation of the receptor stability and activity^[20]. Despite the significance of PDZ domain proteins in the heart (*e.g.*, MAGUK, actinin binding proteins), there is not yet evidence for the specific PDZ-erbB-interacting proteins in cardiomyocytes. The erbB4 receptors, which are endocytosis-impaired, are also regulated through proteolysis, which is mediated by the proteasome system and the alternative transcriptional activity of the cleavable juxtamembrane isoform JMa^[21,22]. These mechanisms either drive the erbB4 protein degradation or induce the nuclear translocation of the JMa intracellular domain. As occurs for the release of the NRG1 active peptides, the release of the erbB4 JMa C-terminal domain is modulated by the activation

of PKC and cleaved by the activity of the tumor necrosis factor- α converting enzyme and of γ -secretase at the plasma membrane^[23]. In the heart, the identified erbB4 protein in cardiomyocytes is the JMb non-cleavable splice variant^[24], which may be proteolytically modulated by the proteasome system. Three PPXY motifs couple erbB4 with WW domain proteins, such as Wwox and ubiquitin ligases, thereby either modulating the transcriptional activity of the c-terminal domain when translocated into the nucleus or promoting the isoform degradation^[25]. Of the two cytoplasmic splice variants CYT1 and CYT2, CYT1 mediates specific interactions with SH2 and WW binding domain proteins (*e.g.*, PI 3'-kinase, ubiquitin ligases)^[26].

Interestingly, *erbB4* polymorphisms and splicing vari-

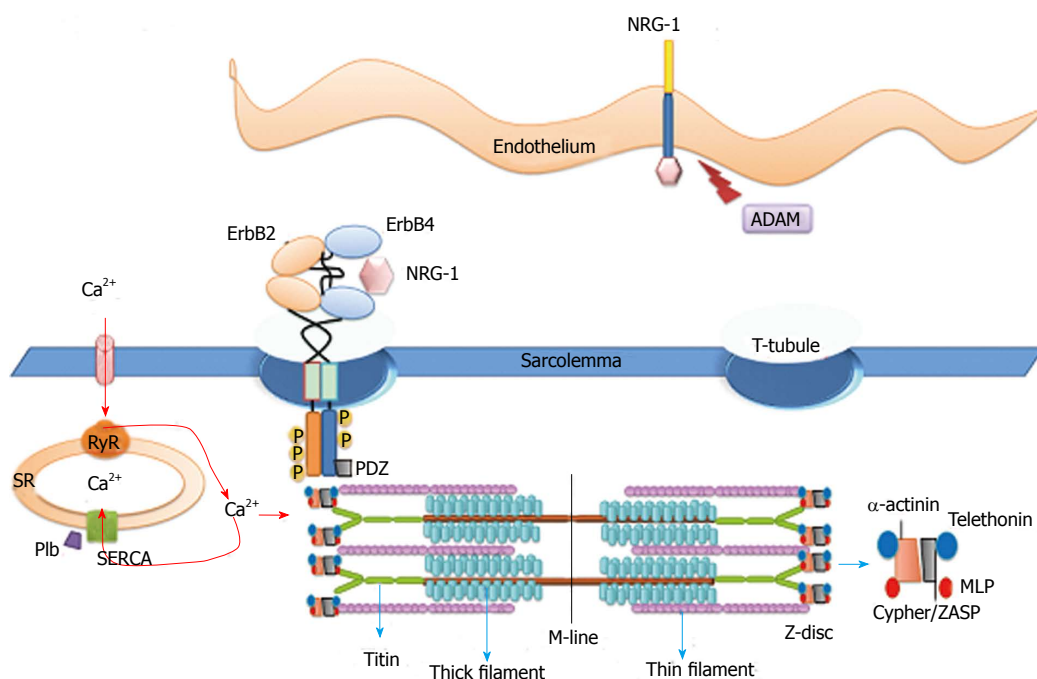


Figure 4 Functional interaction of molecules placed at the T-tubules. The ErbB2 and ErbB4 proteins are localized to the T-tubules. This compartment of the sarcolemma provides specific sites for functional interactions with molecules at the sarcoplasmic reticulum and at the myofibril Z-band. Molecular interactions at the T-tubules and at the intercalated discs provide the electric-contraction coupling of the myocardium. Scheme of the sarcomeric units of thin and thick filaments are anchored by titin and actin at the Z-band via α -actinin scaffold protein complex. Muscle LIM domain protein (MLP), telethonin (T-cap) and PDZ and LIM domain protein (ZASP). PDZ: Postsynaptic density 95, disc large and zonula occludens-1; ADAM: A desintegrin and metalloproteinase; SERCA: Sarcoplasmic reticulum calcium ATPase; Plb: Phospholamban; RyR: Ryanodine receptor; SR: Sarcoplasmic reticulum.

ants of the *erbB4* gene and, more recently, of *Nrg-1* and *erbB2* have been linked to the pathogenesis of non-neoplastic disorders^[27-30]. However, further experimentation is still needed to address the regulation and specific functions of isoforms in cardiovascular, psychiatric, and other diseases.

ERBB REQUIREMENT IN POSTNATAL AND ADULT HEART

The clinical implication of the NRG1 signaling in cardiology results from the increased incidence of dilated cardiomyopathies in a subpopulation of breast tumor patients undergoing the combined administration of anthracycline chemotherapy and humanized antibodies against erbB2 protein^[4]. The cardiac effect of the antibodies (*i.e.*, trastuzumab, pertuzumab), which are species specific and do not cross-react with the mouse protein, were experimentally assessed through the ventricular cardiomyocyte-specific deletion of either the *ErbB2* or the *ErbB4* gene in mice. A ventricular-specific mutation in either of these genes caused dilated cardiomyopathy during adulthood (Figure 3)^[31-33]. These murine models were useful for demonstrating the cardiomyocyte-autonomous requirement of erbB2/erbB4 during the postnatal remodeling of the myocardium. In agreement with the role of the NRG1-erbB2/erbB4 pathway to prevent ventricular dilation during remodeling, the lapatinib-mediated inhibition of erbB2 phosphorylation in mice resulted in a pathological pregnancy-related dilation of the ventricular

chambers that occurred without apparent apoptotic cell death (Table 1)^[34].

The association of changes in the NRG1/erbB pathway with disease was also suggested by the downregulation of both erbB2 and erbB4 expression during the pathologic remodeling of the myocardium in rodents under pressure overload and in humans with a failing myocardium^[35]. However, it is plausible that a different isoform than the normally expressed JMb could be expressed in the failing myocardium. In this regard, the human erbB4 JMa CYT1 isoform manifested a similar activity to the endogenous erbB4 JMb isoform in cardiac morphogenesis in transgenic mice^[36]. A different role than the classical activity of transmembrane tyrosine kinase receptor is displayed by the cleavable erbB4 isoform, which acts as a transcriptional co-activator or co-repressor^[37]. The nuclear localization of the full-length erbB4 protein has recently been observed in cultured adult rat cardiomyocytes under the stress conditions of cell isolation, and the protein was suggested to participate in DNA damaging processes^[38].

Subcellular localization of erbB2/erbB4

Clues about a local cardiomyocyte effect of NRG1 on the maintenance of the myocardial architecture may arise from the subcellular localization of the receptors. The erbB2/erbB4 proteins accumulated within the T-tubule membrane system of cardiomyocytes and the intercalated discs (ID) (Figure 4)^[33].

The relevance of the receptor localization is that the

T-tubules place membrane molecules in close apposition with the myofibril Z-disc, thereby providing a specific context for the functional interactions among molecules at the plasma membrane, sarcoplasmic reticulum (SR) and the myofibril Z-disc. The ID are highly specialized Z-disc structures at the cell-cell contact that provide the anchorage of the sarcomeres to the membrane and place the connexin-43 gap junctions, assuring electric transmission and rhythmic contraction of cardiomyocytes.

Concerning the erbB subcellular context, it is speculated that NRG1 may act on cues at the cytoskeletal pathways that are required for myocardial remodeling. A connection to the cytoskeleton may be required for the feedback modulation of NRG1 signaling with wall stress and contractile parameters during cardiac chamber morphogenesis^[39].

CARDIOPROTECTIVE AND REGENERATIVE ACTIVITIES

The *in vivo* administration of NRG1, under different conditions of mouse cardiac pathology, contributed to the amelioration of ventricular contraction (*e.g.*, reduced left ventricular end systolic dimensions, increased ejection fraction). In these experiments, the NRG1-mediated functional performance of the ventricular chambers was correlated with the increased phosphorylation of the myosin regulatory light chain (RLC) (Table 1)^[40]. However, the administration of NRG1 in knockout mice for the myosin light chain kinase (*Mlk*) gene improves cardiac function without increasing the phosphorylation level of RLC^[41], thus implicating additional mechanisms for contractile improvement.

In cultured cardiomyocytes, NRG1 exerts a negative inotropic effect through either NOS activity or the activation of the muscarinic response (Table 1)^[42,43]. Both nitric oxide and active muscarinic receptors modulate the inotropic response to beta-adrenergic stimulation, which may result in an improved fractional shortening. Indeed, there is a general lack of evidence for either a direct NRG1-mediated inotropic effect or an induced change in calcium handling or in myofilament calcium sensitivity that may provide a molecular explanation for the NRG1-mediated enhancement of cardiac systolic function.

Additional repair activities have been suggested through the induced proliferation of cardiomyocytes in a murine model of cardiac infarction. In this setting, NRG-1/erbB4 was shown to induce mononucleated cardiomyocytes to proliferate, thereby contributing to the cardiac repair mechanisms in one-week-old myocardial infarction without affecting the level of apoptotic cell death (Table 1)^[44]. This activity is particularly interesting for the renewed research of the cardiomyocytes' re-entry into the cell cycle and the use of stem cells to re-populate the injured myocardium^[45-47]. In this regard, pluripotent cells may also repair damage myocardial areas through the secretion of relevant growth factors^[48].

The evidence for the essential role of NRG1-erbB2/

erbB4 prompted an evaluation of the safety and efficacy of NRG1 administration in patients with chronic heart failure and focal ischemia (Phase I NCT01258387, Phase III NCT01439893, NCT01541202 trials)^[49,50]. The panel of activities displayed upon NRG1 administration has been well reviewed^[51] and indicates a pleiotropic effect on cardiac muscle and vascular cells^[51]. The ultimate contribution of each identified NRG1-mediated activity on cardiac performance remains to be defined. Moreover, the employment of NRG1 for the treatment of cardiac dysfunction still requires a mechanistic understanding of how this signal exerts its effect as well as which critical molecular targets of this pathway affect cardiac remodeling.

MECHANISMS OF CARDIOTOXICITY: THE ROLE OF NRG1/ERBB SIGNALING

The evidence of the critical activity of NRG1 towards anthracycline cardiotoxicity led to improvements in the clinical management of administering chemotherapy and antibodies against erbB2. Further investigation is required to understand the molecular link of a NRG1 deficiency and exacerbated cardiotoxicity. The individual therapies with either antibodies against erbB2 or anthracyclines exert a cardiotoxic effect at a lower incidence rate compared to the combined treatment. Chemotherapy with anthracycline derivatives may result in both immediate and delayed cardiomyocyte toxic events. The induction of the oxidative stress response that underlies anthracycline cardiotoxicity has been related to cellular apoptosis and necrosis as the mechanism of toxicity. However, employing antioxidants in radiation- or chemotherapy-treated patients resulted in an unclear improvement in cardiac function. These results led to the conclusion that oxidative stress may be viewed as a two-way process by which radical oxygen species (ROS) mediate tumor cell death and promote cell survival through the degradation of anthracyclines^[52]. In addition, clinical studies aimed to reduce the oxidative and inflammatory process during ischemic dilated cardiomyopathy and chronic heart failure have not yet provided a therapeutic strategy because of dissimilar explanations among the trial results^[53]. It is therefore likely that ROS-independent mechanisms may play a more important role in the doxorubicin-induced myocardial damage than previously evaluated^[54].

Direct evidence for the role of NRG1 against anthracycline cardiotoxicity was provided by the protective activity of NRG1 against doxorubicin-mediated myofibril disarray and in preventing the toxic degradation of troponins in cultured cardiomyocytes (Table 2)^[55,56]. The cardioprotective activity of NRG1 was also inferred *in vivo* by the doxorubicin-aggravated contractile dysfunction in heterozygous NRG1 mutant mice and by the exacerbated cardiac chamber dilation in the ventricular-specific erbB4-knockout mice (Table 2)^[57,58].

The serum level of both the cardiac troponins (*e.g.*, cTnI) and brain natriuretic peptide (BNP) are relevant markers for the follow-up of acquired cardiac pathology

Table 2 Biological effects of combined neuregulin-1/erbB signaling and anthracyclines

Experimental system	Monitored response	Ref.
Cultured cardiomyocytes		
NRG-1 administration	Prevented myofibril disarray	[55]
	Attenuated troponin degradation	[56]
Animal models		
NRG1-deficiency/doxorubicin (NRG1 heterozygous mouse)	Induced heart failure	[57]
NRG1-deficiency/doxorubicin (Ventricular specific-erbB4-KO)	Deregulated Protein Homeostasis Autophagic vacuolization	[58]

Summary of effects mediated by anthracycline-induced toxicity and by neuregulin-1 (NRG1)-erbB2/erbB4 signaling in *in vitro* system and animal models. The NRG-1 activities on cardiomyocyte survival, myofibril organization and ventricular homeostasis were assessed based on the exogenous administration of NRG1 or by the outcome of an impaired signaling as discussed throughout the article.

Table 3 Cardiac phenotypic modifications

Morphology	WT	KO	WTD	KOD
Young adult (1 mo)				
Heart/body weight (mg/g)	5.2 ± 0.4	5.3 ± 0.6	5.2 ± 0.5	6.2 ± 0.8 ^a
Body weight (g)	17.4 ± 2.0	17.3 ± 2.2	17.3 ± 2.0	17.2 ± 1.5
Adult (3 mo)				
Heart/body weight (mg/g)	5.6 ± 0.6	7.0 ± 0.8 ^d	5.4 ± 0.7	6.7 ± 0.8 ^d
Body weight (g)	30.1 ± 2.5	30.3 ± 2.2	29.4 ± 2.2	28.4 ± 2.2
Cardiotoxic gene groups				
Dilation (ratio)	-	3	1.8	2.8
Hypertrophy (ratio)	-	9.9	3.3	6.5
Damage (ratio)	-	3.7	3.7	3.6
Cell death/necrosis (Ratio)	-	3.0	4.3	3.1
Measured activity				
Caspase 3 (arbitrary units)	0.25 ± 0.17	0.44 ± 0.17	0.84 ± 0.09 ^a	0.35 ± 0.07
Autophagic vacuolization (n°)	0.07 ± 0.1	0.35 ± 0.3	0.2 ± 0.2	2.4 ± 2.1 ^a
Serum cTnI (mg/mL)	0.15 ± 0.1	3.6 ± 1.5	0.7 ± 0.2	14.5 ± 4.2 ^a
LVDP (mmHg)	109.2 ± 7.1	42 ± 8.2 ^a	94.5 ± 5.5	31.5 ± 5.4 ^a
Tau ½	37.4 ± 1.9	38.1 ± 4.9	36.1 ± 3.1	34.6 ± 2.9

Morphological and biochemical modifications studied in the aggravated cardiotoxic condition of the doxorubicin-treated-ventricular specific erbB4-KO mouse model. Summary of hypertrophic-associated morphological changes determined in mouse at the age of 1 and 3 mo, group of differentially expressed genes clustered into a wide-range of cardiotoxic conditions, biochemical activities associated to cell death and of physiological systolic and diastolic parameters are represented as discussed in the text. ^a*P* < 0.05 *vs* WT, ^d*P* < 0.01 *vs* WT and WTD. WTD: Doxorubicin-treated wildtype; KOD: Doxorubicin-treated erbB4-KO; WT: Treated wildtype.

in trastuzumab- and anthracycline-treated patients^[59,60]. Indeed, the detection of the cTnI and BNP serum levels was useful for validating the exacerbated cardiotoxicity displayed by an injection of doxorubicin in the ventricular-specific erbB4-knockout mouse. The hypertrophic hallmark, *i.e.*, the natriuretic peptide of the atrial type, was expressed at a relatively low level in doxorubicin-treated wildtype (WTD) animals and at intermediate levels in the doxorubicin-treated erbB4-KO (KOD), compared to the robust expression in erbB4-KO^[33,58]. The expression of BNP was at a similar level in the erbB4-KO and

in both WTD and KOD mice compared to the wildtype. A contrasting finding between WTD and treated KOD mice was the differential expression of both a group of genes related to oxidative stress and molecules of the apoptotic-death pathway that underlined the WTD (Table 3)^[58]. The administration of doxorubicin to erbB4-KO mice led to the synergistic deregulation of the ubiquitin-proteasome system. The observed downregulation of the IGF-I/PI3³-Kinase axis, which may respond to lower levels of PPAR^[61] or to a deficient activity of CREB under the control of NRG1^[62], may represent a potential mechanism acting on the deregulation of the ubiquitin-proteasome system in erbB4-KOD hearts.

The deregulated group of genes of the ubiquitin-proteasome system was characterized by the upregulation of ubiquitin-ligase, which induced large protein aggregates in cardiomyocytes within the doxorubicin-treated erbB4-KO. Autophagic vacuolization, which is the recommended term for the cellular appearance of large ubiquitin-positive protein aggregates^[63], resulted in a 7-fold increase of affected cardiomyocytes relative to the non-treated erbB4-KO (Table 3). The perturbation of the ubiquitin-proteasome system induced by a genetic modification in mice led to cardiomyocyte necrosis and cardiac chamber dilation^[64]. Moreover, the level of protein ubiquitination was documented as a useful predictor of myocardial deterioration in patients to follow cardiac transplantation^[65]. The monitoring of necrotic cardiomyocytes, by the determination of the cTnI serum level, is a highly sensitive cardiotoxic marker that is employed in the follow-up of breast tumor patients undergoing trastuzumab and chemotherapy^[59,60].

A search for biomarkers in the early detection of doxorubicin cardiotoxicity in both heart and peripheral blood mononuclear cells through the determination of differential gene expression indicated that the most significant group of genes was represented by changes in the canonical NRF2-oxidative stress response pathway, protein ubiquitination and the PI3³K/AKT signaling pathway^[66]. Collectively, these results extend our current knowledge by demonstrating that the impaired NRG1 response in erbB4-KO hearts to doxorubicin toxicity has a net result of the induced autophagic vacuolization of cardiomyocytes, which is consistent with the association of abnormal protein homeostasis with a severe cardiac

disorder.

CONCLUSION

The NRG1/erbB pathway is critical for the maintenance of the myocardial structure in the adult heart, and moreover, impaired NRG1 signaling exacerbates anthracycline-mediated cardiotoxicity. The accumulated evidence indicates that NRG1 displays a panel of protective and repair activities in the heart during the lifespan of an individual. In this context, an impaired NRG1 signaling sensitizes the heart to the toxicity of anthracycline. There is an ongoing search for drugs and immunotherapies that can inhibit the erbB receptors implicated in tumorigenesis, which may also display an iatrogenic effect in the heart. The individual treatment with either anthracycline derivatives or the induced deficiency in the NRG1 pathway displayed different gene expression profiles in experimental murine models. The doxorubicin-treated hearts were characterized by an oxidative stress response, which may induce cardiomyocyte apoptosis. The sensitization of the NRG1-deficient heart to the anthracycline toxicity resulted in a potentiated deregulation of the ubiquitin-proteasome system, with a net result of the autophagic vacuolization of cardiomyocytes.

Altogether, the NRG1 activities that affect the myocardial architecture and homeostasis await a mechanistic understanding of how NRG1 modulates remodeling and thereby prevents ventricular dilation. Continuous research in this area will provide critical molecules and targets that may help in the design of diagnostic tools and therapeutic.

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Assessment of coronary artery disease using coronary computed tomography angiography and biochemical markers

Gitsios Gitsioudis, Hugo A Katus, Grigorios Korosoglou

Gitsios Gitsioudis, Hugo A Katus, Grigorios Korosoglou, Department of Cardiology, University of Heidelberg, 69120 Heidelberg, Germany

Author contributions: All the authors solely contributed to this paper.

Correspondence to: Grigorios Korosoglou, MD, Department of Cardiology, University of Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany. gkorosoglou@hotmail.com

Telephone: +49-6221-564130 Fax: +49-6221-565513

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Abstract

Chronic inflammatory mechanisms in the arterial wall lead to atherosclerosis, and include endothelial cell damage, inflammation, apoptosis, lipoprotein deposition, calcification and fibrosis. Cardiac computed tomography angiography (CCTA) has been shown to be a promising tool for non-invasive assessment of these specific compositional and structural changes in coronary arteries. This review focuses on the technical background of CCTA-based quantitative plaque characterization. Furthermore, we discuss the available evidence for CCTA-based plaque characterization and the potential role of CCTA for risk stratification of patients with coronary artery disease.

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Key words: Atherosclerotic plaque composition; Quantification analysis; Multi-slice cardiac computed tomography; Biomarkers

Core tip: This review gives an overview of the current status of noninvasive assessment of coronary artery disease (CAD) and the ability of cardiac computed tomography angiography (CCTA) and cardiac biomarkers

for the diagnostic classification and risk stratification of patients with suspected and known CAD. Since all techniques described herein are available in the clinical routine and are associated with an acceptable time spent the translation to the clinical realm appears promising. Focusing on CCTA-based quantitative plaque characterization we herein present the (1) available evidence; (2) comparison with other techniques of plaque characterization; and (3) the value of "bio-imaging" for the risk stratification of patients with CAD.

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INTRODUCTION

Sudden vessel occlusion as a consequence of atherosclerotic plaque rupture with subsequent coronary artery thrombosis is the most common cause of acute myocardial infarction (AMI) and sudden cardiac death in the industrialized world^[1]. Conventional X-ray coronary angiography still remains the gold standard for detection of coronary artery disease (CAD). However, this technique is invasive and provides limited information on the composition of atherosclerotic plaque^[2]. Coronary computed tomography angiography (CCTA) on the other hand, is a very fast evolving and in the meanwhile well-established non-invasive technique for the visualization of both coronary artery lumen narrowing and coronary calcification^[3]. In addition, CCTA with the help of commercially available software tools provides objective and quantitative assessment of atherosclerotic plaque composition^[4-6].

Based on recent developments with CCTA hardware

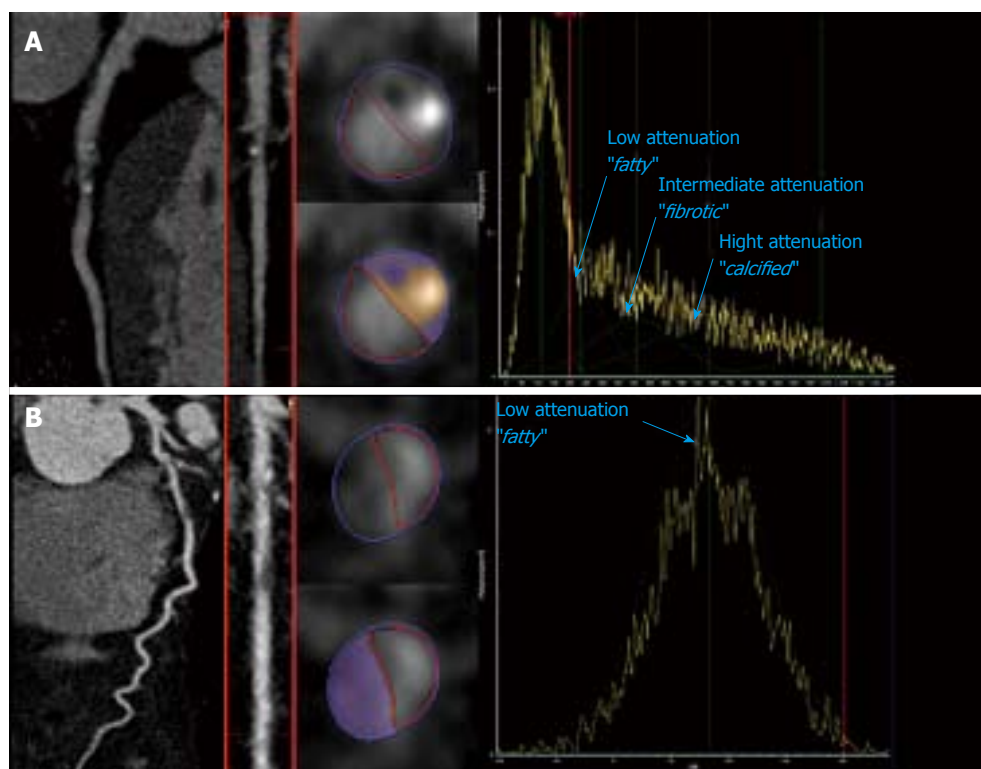


Figure 1 Representative example of (A) a partially calcified and (B) of a non-calcified atherosclerotic coronary plaque with the corresponding Gaussian curves, respectively for different plaque components (lipid-rich, fibrotic and calcified).

and software technologies, including iterative reconstruction algorithms, a substantial reduction in radiation exposure and improvement of image quality could be achieved^[7-11]. In addition, dedicated post-processing tools constituted major steps towards the reliable and quantitative assessment of atherosclerotic plaque composition^[12-17].

The growing body of evidence for the prognostic value of CCTA-based plaque characterization underscores its potential for implementation in the clinical realm. In this regard, features indicating plaque vulnerability include a large necrotic core, thin fibrous cap and positive vessel remodeling^[6,18-22]. The early and non-invasive detection of such vulnerable rupture-prone atherosclerotic lesions remains a major challenge in patient care.

DATA ON THE FEASIBILITY OF CCTA-BASED CORONARY PLAQUE CHARACTERIZATION

First generation CCTA scanners offered limited ability for the reliable detection of coronary lesions due to technical limitations, including limited spatial and temporal resolution, and partial volume effects caused by coronary calcifications. With the development of 256- or even 320-slice multi-slice CT-scanners however, faster gantry rotation speed, Z-direction focal-spot sampling and spherical detector design could overcome these limitations, offering high isotropic spatial resolution of

approximately 400-600 μm and a temporal resolution of approximately 83-175 ms^[7,9,23-26].

Current SCCT guidelines introduced a scheme for the qualitative characterization of different plaque types for clinical reporting^[27]. In general, the percentage of calcium content is < 20% in non-calcified plaque, between 20% and 80% in mixed plaque and > 80% in calcified plaque. The reproducibility of this qualitative assessment (calcified, non-calcified, mixed plaques) has been shown to be good for both intra- and inter-observer agreements with more than 88%^[28,29]. The accuracy of this qualitative plaque characterization approach has been validated by virtual histology-intravascular ultrasound (VH-IVUS) for different plaque types^[30].

Others and we showed the feasibility and practicability of semi-automated and automated post-processing software tools for the quantitative assessment of atherosclerotic coronary plaque size and composition in patients undergoing CCTA for clinical reasons^[17,31-33]. This volumetric approach allows for assessment of (1) total plaque volume, (2) plaque composition (distribution of (non-) calcified content) and (3) maximum, mean and minimum plaque intensities in hounsfield units (HU). Hoffman *et al.*^[33] showed that limits of agreement are approximately 60% for small volumes (10 mm³) and 28% for larger volumes (100 mm³). According to the tissue specific attenuation properties, three different plaque components can potentially be distinguished, including: (1) lipid-rich (14-70 HU); (2) fibrotic (71-150 HU); and (3) calcified components (> 150-200 HU)^[14]. Lipid and fi-

Table 1 Table summarizing the current key studies on comprehensive “bio-imaging” with coronary computed tomography and biomarkers in presumably stable coronary artery disease patients

Ref.	Biochemical markers	CT scanner	Number of patients	Results
Laufer <i>et al</i> ^[59]	hsTnT	64-sl. MDCT	615	Even mild CAD is associated with hsTnT levels in symptomatic patients
Korosoglou <i>et al</i> ^[58]	hsTnT	≥ 64-sl. MDCT	124	hsTnT is associated with the extend of positive remodeled NCP. Only weak association was detected for hsCRP
Blaha <i>et al</i> ^[68]	hsCRP	4-sl. MDCT	6762	hsCRP was not associated with coronary artery calcification
Duivenvoorden <i>et al</i> ^[70]	hsCRP, MPO, and others	¹⁸ FDG-PET/CT	130	MPO levels are associated with carotid plaque inflammation
Andrassy <i>et al</i> ^[62]	HMBG-1	256-sl. MDCT	152	HMBG1 is associated with the composition and extend of atherosclerotic plaques
Nakazato <i>et al</i> ^[64]	LDL, HDL, TC	≥ 64-sl. MDCT	4575	Presence and extend of NCP are associated with high non-HDL level
Voros <i>et al</i> ^[63]	ApoB, HDL, LDL	64-sl. MDCT IVUS/VH	60	ApoB and small HDL particles are associated with larger plaque burden and more NCP plaque. Larger HDL and pre-b2-HDL particles are associated with plaque burden and less NCP

CAD: Coronary artery disease; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; ApoB: Apolipoprotein B; TC: Total cholesterol; hsTnT: High-sensitive Troponin T; hsCRP: High-sensitive C-reactive protein; MPO: Myeloperoxidase; MDCT: Multi-slice computer tomography.

brotic plaque components are often summarized as “non-calcified”. However, there is still a lack of a uniform attenuation cut-off values defining these tissue qualities due to overlapping attenuation intervals. Figure 1 shows representative examples of a (A) non-calcified and (B) of a partially calcified atherosclerotic coronary plaques with the corresponding Gaussian curves, respectively for different plaque components.

Previous *ex vivo* studies compared CCTA-based plaque characteristics with histopathology^[34-36]. In this regard, 16- and 64-slice CCTA provided precise detection of calcified lesion, while its accuracy for the differentiation between lipid-rich and fibrotic components was lower^[37-39]. Further experimental studies are now warranted to reevaluate the potential of 256- and 320-slice scanners in this context.

VIRTUAL HISTOLOGY-INTRAVASCULAR ULTRASOUND

VH-IVUS with radiofrequency backscatter analysis is the clinical gold standard technique for the visualization of coronary vessel wall morphology^[40,41]. In *ex vivo* studies of coronary arteries, IVUS has been shown to successfully identify plaque features as regional calcification, lipid-rich necrotic cores and fibro-fatty plaques with high accuracy^[42-44]. From a clinical point of view, the PROSPECTIVE trial could show the prognostic impact of IVUS-based plaque characterization in patients with acute coronary syndromes^[21]. In contrast to CCTA, VH-IVUS enables for detailed measurement of fibrous cap thickness and for the detection of thin-cap fibroatheromas (TCFA)^[38,45]. Pundziute *et al*^[40] showed that 32% of partially calcified plaques in CCTA were characterized as TCFA by VH-IVUS.

However, there are still some limitations both during IVUS data acquisition and in the post-processing raw data handling^[46]. In addition, the assessment of the entire coronary tree requires a 3-vessel catheter-based interrogation, which may involve additional risks for the

patients^[21]. In this regard, CCTA would be a valuable non-invasive alternative to IVUS, especially in light of the good correlation of the 2 techniques in terms of plaque composition assessment^[14,32,38,47-49].

OPTICAL COHERENCE TOMOGRAPHY AND NEAR INFRARED SPECTROSCOPY

Other intravascular imaging techniques like optical coherence tomography (OCT) and near infrared spectroscopy (NIS) have also been applied for the assessment of coronary plaque composition. OCT which is the light analogue of IVUS enables for a resolution of 10-20 μm, which is about 10 times higher than that provided by IVUS. OCT detects erosions and can also differentiate between red and white thrombus^[50]. However, OCT cannot visualize vessel wall structures under the condition of blood flow, has limited penetration depths of 1-2 mm, and is therefore not appropriate for deeper imaging of blood vessels^[51]. Despite continuing improvements in the performance of both IVUS and OCT, their use has been mostly limited to structural imaging so far. On the other hand, near infrared spectroscopy (NIS) belongs to a different class of imaging methods which measures absorption spectra from blood vessels in order to assess lipid content^[51,52]. However, additional experimental and clinical data are required to assess the methodological reliability and to define precise clinical applications with this technique. Finally, the detection of lipid subtypes, such as oxidized low-density lipoprotein (ox LDL) is still limited using NIS.

RISK STRATIFICATION USING CCTA AND BIOCHEMICAL MARKERS

The primary adverse outcome of CAD is acute myocardial infarction (AMI) and sudden cardiac death. Therefore, there is a great need for robust diagnostic algorithms, which may include cardiac biomarkers and non-invasive imaging techniques, for the risk stratification

of patients with subclinical or presumably stable CAD. In this regard, the detection of rupture-prone coronary plaques or of elevated cardiac troponins may help the classification of patients with presumably low risk *vs* those with high-risk, aiding in the guidance of pharmacologic and interventional treatment strategies. Non-invasive assessment of functional wall motion analysis by dobutamine stress cardiac magnetic resonance imaging (MRI) or stress echocardiography has also been shown to identify patients at high risk for future cardiac events^[53,54]. However, in contrast to CCTA these imaging modalities provide no information on coronary artery pathologies and plaque composition.

Several cardiovascular biomarkers are well established in clinical routine to complement clinical assessment and 12-lead ECG in the diagnosis, risk stratification, triage, and management of patients with suspected acute coronary syndrome (ACS). Especially cardiac troponins were shown to aid the diagnostic classification and risk stratification of patients with ACS^[55-57]. Recently others and we could show an association between CTA atherosclerotic plaque characteristics and small blood level troponin increases in patients with stable CAD^[58,59], which could be explained by chronic clinically silent rupture of non-calcified plaque with subsequent microembolisation. In an experimental setting, high mobility group box 1 (HMBG1) protein was found to be a critical mediator of acute ischemic injury, predicting adverse outcomes after myocardial infarction^[60,61]. In addition, we could show that HMBG1 serum levels are associated with coronary calcification and with non-calcified plaque composition in patients with suspected or known stable CAD^[62].

Incorporation of ox-LDL transforms macrophages into foam cells, which built the core of atherosclerotic plaques. In this regard, the presence and extent of non-calcified plaques are associated with high non-HDL, which suggest a relationship between lipid profile and plaque composition^[63,64].

CRP was initially supposed to be a causal player for atherosclerotic plaque development and inflammation^[65]. However, further basic science research has questioned a direct atherogenic mechanism^[66,67]. Others and we could show that serum levels of hsCRP are only weakly correlated with plaque composition and coronary artery calcification and largely determined by the presence of risk factors^[58,68,69]. More specific markers of inflammation could provide a stronger association with plaque formation and atherosclerotic inflammation. In this regard, the dal-PLAQUE study recently showed that myeloperoxidase levels are associated with carotid plaque inflammation, which was assessed using 18F-fluorodeoxyglucose positron emission tomography/computed tomography^[70]. An overview of the most interesting studies in the area of comprehensive “bio-imaging” using cardiac computed tomography and biomarkers are presented in Table 1.

Several CCTA outcome studies on the other hand, have assessed the prognostic value of plaque burden and plaque morphology in both symptomatic and asymp-

tomatic cohorts^[18,71-74]. The value of risk assessment in patients with CAD using a CCTA-based semi-automated plaque assessment has been recently shown^[6]. Ongoing studies now investigate the potential complementary value of high-sensitive Troponin T (hsTnT) and quantitatively assessed coronary plaque burden for the risk stratification of patients with intermediate likelihood for CAD.

CONCLUSION

Imaging of coronary artery disease using CCTA is a feasible and robust approach for non-invasive plaque characterization. Growing body of evidence exists for the ability of CCTA based quantitative plaque characterization for the prediction of clinical outcome in patients with suspected or known coronary artery disease.

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Lipid profile in children with coronary artery disease in Sindh, Pakistan

Saira Baloch, Bikha Ram Devrajani, Mohsin Ali Baloch, Mohammad Ali Pir

Saira Baloch, Bikha Ram Devrajani, Medical Research Center, Liaquat University of Medical and Health Sciences, Sindh 75000, Pakistan

Mohsin Ali Baloch, Faculty of Pharmacy, University of Sindh, Jamshoro 75000, Pakistan

Mohammad Ali Pir, Department of Community Medicine and Public Health, Liaquat University of Medical and Health Sciences, Sindh 75000, Pakistan

Author contributions: Baloch S and Devrajani BR collected, analyzed and interpreted the data, and designed the study; Baloch MA and Pir MA participated in the collection of data.

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Correspondence to: Saira Baloch, Assistant Professor, Medical Research Center, Liaquat University of Medical and Health Sciences, Jamshoro, Sindh 75000, Pakistan. saira.baloch@lumhs.edu.pk

Telephone: +92-22-921331617 Fax: +92-22-9213315

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CONCLUSION: CAD risk factors are significant regarding abnormal lipid levels. Genetic tendency seems to be important in the development of CAD in children.

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Key words: Serum; Lipid profile; Coronary artery disease; Children; Sindh

Core tip: It is well known that cholesterol accumulates in the coronary wall and conditions of blood pressure are recurrently connected with coronary artery disease in early adult life.

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Abstract

AIM: To evaluate lipid profile in children with coronary artery disease (CAD) in Hyderabad, Sindh, Pakistan.

METHODS: The study included 100 children (6-15 years), of which 43 were children of young parents (one or both) with recognized CAD, while the other 57 were children with no evidence of CAD (controls). All were evaluated for fasting blood lipid profile. Blood samples were collected from patients with CAD and healthy controls and analysis of the levels of lipid profile were carried out using a kit method on Microlab 300.

RESULTS: Children with CAD had significantly higher levels of total serum cholesterol and triglycerides and decreased levels of high density lipoprotein and low density lipoprotein compared to children in the control group. Systolic and diastolic blood pressures were significantly higher, without any significant difference.

INTRODUCTION

Coronary artery disease (CAD) is one of the main causes of mortality and morbidity in Pakistan. It is assessed that in the future these diseases will constitute major public health problems. Propensity CAD risk factors stimulate the progression of main and conditional CAD risk factors that cause CAD. Numerous lifestyle aspects with diet, environmental factors and genetic predisposition affect the outcome and development of atherosclerosis and thrombosis^[1]. The progression of risk factors and their association with the manifestation of CAD has been developed from worldwide prospective epidemiological studies. These studies have revealed a constant correlation among characteristics examined in healthy individuals with the consequent prevalence of CAD^[2]. The results have drawn attention to the status of risk factors in formative outcomes^[3] and heterogeneity of CAD

Table 1 Lipid Profile in children with coronary artery disease and control group

Variables	Children with CAD	Control group
Total cholesterol	62.1 ± 41.1	44.6 ± 15
Triglycerides	45.3 ± 21.2	29.4 ± 17
HDL	15.1 ± 13	19.2 ± 22
LDL	12.3 ± 0.2	17.2 ± 12

CAD: Coronary artery disease; HDL: High density lipoprotein; LDL: Low density lipoprotein.

patients. Family history of CAD is highly associated with disease occurrence^[4]. Hypertension^[5] frequently has a correlation with CAD. Increased serum cholesterol levels^[6] are associated with the risk of CAD and decreased levels of low density lipoprotein (LDL) and high density lipoprotein (HDL) are important in the progression^[7] of CAD. Hypertriglyceridemia is known^[8] in the progression of CAD.

It is well known that cholesterol accumulates in the coronary wall and conditions of blood pressure are recurrently connected by CAD in early adult life. Fatty streaks can be noticed in infants by 2-3 mo of age and increase in size and number throughout the first two decades of life^[9-13]. Dyslipoproteinemia with high levels of total cholesterol and LDL and low levels of HDL and family history of early CAD have been demonstrated to be predisposing factors of early CAD^[9-14]. Recently, more emphasis has been laid on the role of lipoproteins than cholesterol alone^[15,16]. The aim of this study is to analyze main lipid and lipoprotein cholesterol spectrum in children with respect to the CAD history of their parents with or without hypercholesterolemia. Although cardiovascular diseases do not manifest until maturity, dyslipidemia risk factors are present in children and remain into old age^[17]. It was suggested that lipid profile levels should be screened in children, providing a procedure to recognize and treat those who are at risk for the progression of CAD^[18,19]. Cardiovascular disease is one of the major problems in Asia but very few studies have been documented about the lipid profile and incidence of dyslipidemia in children, which prove irregular lipid profiles^[20]. These studies also reported the high levels of lipid disorders in children. Therefore, the current study was carried out to detect serum lipid profiles and the prevalence CAD among children.

MATERIALS AND METHODS

The study included 100 children (6-15 years), of which 43 were children with recognized coronary artery disease (CAD) and 57 children with no confirmation of CAD (healthy controls). The case group was selected from patients admitted or visiting the pediatric unit at LUMHS City and Jamshoro Hospital for angiography or medical treatment. The inclusion criterion for the case group was structural CAD diagnosed by echocardiography or angiography, and the inclusion criteria for both groups were

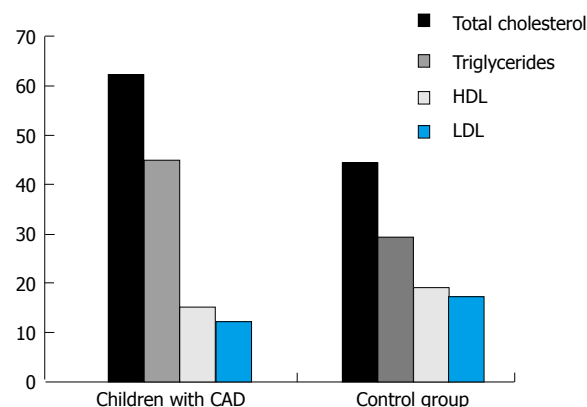


Figure 1 Total cholesterol, triglyceride high density lipoprotein and low density lipoprotein levels in children with coronary artery disease compared to the control group. HDL: High density lipoprotein; LDL: Low density lipoprotein; CAD: Coronary artery disease.

not having chronic liver or kidney disease which may disturb lipid profile levels. The children and their parents received complete justifications about the study (including procedural details and sampling) and informed consent was obtained from the parents before the beginning of the study. The echocardiographic studies comprised using an echocardiographic machine. All the measurements were performed by one pediatric cardiologist. Ten mL blood samples from coronary artery disease patients and healthy control subjects were collected and serum was separated and immediately levels of the lipid profile were analyzed using a kit method on Microlab 300. Excel and SPSS.15 were used for data analysis.

RESULTS

Table 1 and Figure 1 show mean serum levels of lipid profile in the CAD and control groups. The results showed a significant increased level of total cholesterol and triglycerides and a decreased level of HDL and LDL compared to the controls, with $P < 0.001$.

DISCUSSION

The genetic factor is supposed to be the leading factor when CAD presents early in life. Several studies have documented the association between cholesterol levels and prevalence of CAD^[21,22]. The association between CAD and levels of cholesterol is complex to estimate in children because clinically significant CAD does not happen. In the current study, the children of parents with CAD have a significant occurrence of hyperlipidemia and there is an association between lipid profile levels of children^[23-26]. It was reported^[27] that 72 children whose ancestors had myocardial infarction had increased levels of cholesterol; however, there was no significant difference in levels of triglyceride. It was reported that there was an association among lipid profile levels of parents and their children with total cholesterol levels. This study is similar to other studies^[27].

Increased levels of serum cholesterol, triglycerides and LDL are several of the significant factors in these patients. It was reported that hypercholesterolemia is common in children of parents with recognized hypercholesterolemia and symptomatic coronary artery disease. An increased total cholesterol along with HDL ratio influences primary coronary artery disease^[28]. This ratio in the current study of high risk children was significantly more increased than the ratio given by earlier workers^[29]. It has been revealed that the entire risk factor separately enhances the risk of coronary artery disease by 5 to 10 times compared with having no risk factors.

The present study observed the lipid profile in children with CAD compared to the control group. High levels of cholesterol and triglyceride and low levels of HDL and LDL in children with CAD were found. Our results conclude that it is useful to monitor the lipid profile of children of parents with coronary artery disease. Children of parents with CAD and hyperlipidemia are at high risk of progression to premature atherosclerosis and need lipid profile assessment monitoring.

The lipid profile of children diagnosed with intermittent major risks can be taken to reduce these risks. Further studies with greater sample numbers are necessary to confirm these findings.

COMMENTS

Background

Coronary artery disease (CAD) patients are at risk for poor nutritional status. The entire measures lead to life intimidating problems and are predictive factors.

Research frontiers

Lipid profile disturbances between the patients were compared with healthy subjects. This study is designed with the objective of investigating the similarities and differences in patients with CAD, clinically and metabolically.

Innovations and breakthroughs

Children with CAD had significantly higher levels of total serum cholesterol and triglycerides and decreased levels of high density lipoprotein and low density lipoprotein compared to the control group. Systolic and diastolic blood pressures were significantly higher, without any significant difference.

Applications

By understanding the lipid profile in CAD patients and controls for the progression of future remedial guidelines, this study may need to be sustained in a further extensive manner at different nursing homes.

Peer review

The authors made a good effort to analyze the lipid profile in children whose parents are known to have coronary artery disease.

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Is cardiac MRI an effective test for arrhythmogenic right ventricular cardiomyopathy diagnosis?

Santhi Chellamuthu, Alyson M Smith, Steven M Thomas, Catherine Hill, Peter W G Brown, Abdallah Al-Mohammad

Santhi Chellamuthu, Steven M Thomas, Catherine Hill, Peter W G Brown, Department of Radiology, Sheffield Teaching Hospitals NHS trust, Northern General Hospital, Sheffield S5 7AU, United Kingdom

Alyson M Smith, Abdallah Al-Mohammad, Department of Cardiology, Sheffield Teaching Hospitals NHS Trust, Northern General Hospital, Sheffield S5 7AU, United Kingdom

Author contributions: Chellamuthu S and Smith AM contributed to the acquisition, analysis and interpretation of data, drafting and revision of the article; Thomas SM, Hill C, Brown PWG and Al-Mohammad A contributed to the conception and design of the study, revision of the article for important intellectual content; all authors approved the final version to be published.

Correspondence to: Dr. Alyson M Smith, Department of Cardiology, Sheffield Teaching Hospitals NHS Trust, Northern General Hospital, Herries Road, Sheffield S5 7AU, United Kingdom. smith_alysan@hotmail.com

Telephone: +44-11-42434343 Fax: +44-11-42434343

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Abstract

AIM: To evaluate the referrals with suspected arrhythmogenic right ventricular cardiomyopathy (ARVC) and compare cardiac MR (cMR) findings against clinical diagnosis.

METHODS: A retrospective analysis of 114 (age range 16 to 83, males 55% and females 45%) patients referred for cMR with a suspected diagnosis of ARVC between May 2006 and February 2010 was performed after obtaining institutional approval for service evaluation. Reasons for referral including clinical symptoms and family history of sudden death, electrocardiogram and echo abnormalities, cMR findings, final clinical diagnosis and information about clinical management were obtained. The results of cMR were classified as major, minor, non-specific or negative depending on both functional and tissue characterisation and the cMR results

were compared against the final clinical diagnosis.

RESULTS: The most common reasons for referral included arrhythmias (30%) and a family history of sudden death (20%). Of the total cohort of 114 patients: 4 patients (4%) had major cMR findings for ARVC, 13 patients (11%) had minor cMR findings, 2 patients had non-specific cMR findings relating to the right ventricle and 95 patients had a negative cMR. Of the 4 patients who had major cMR findings, 3 (75%) had a positive clinical diagnosis. In contrast, of the 13 patients who had minor cMR findings, only 2 (15%) had a positive clinical diagnosis. Out of the 95 negative patients, clinical details were available for 81 patients and none of them had ARVC. Excluding the 14 patients with no clinical data and final diagnosis, the sensitivity of the test was 100%, specificity 87%, positive predictive value 29% and the negative predictive value 100%.

CONCLUSION: CMR is a useful tool for ARVC evaluation because of the high negative predictive value as the outcome has a significant impact on the clinical decision-making.

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Key words: Arrhythmogenic right ventricular cardiomyopathy; Cardiomyopathy; Right ventricular; Arrhythmias; Magnetic resonance imaging; Diagnosis; Implantable cardiac defibrillator

Core tip: This study was designed to evaluate the referrals with suspected Arrhythmogenic right ventricular cardiomyopathy (ARVC) and compare the findings of cardiac magnetic resonance imaging (cMR) against clinical diagnosis. Currently the diagnosis depends upon a combination of variety of factors including imaging findings. We evaluated all the referrals in our institution over a 4-year period and found a high sensitivity and specificity of cMR for ARVC diagnosis. We have concluded that cMR is a very useful tool for ARVC evalua-

tion because of the very high negative predictive value as the outcome has a significant impact on the clinical decision-making.

Chellamuthu S, Smith AM, Thomas SM, Hill C, Brown PWG, Al-Mohammad A. Is cardiac MRI an effective test for arrhythmogenic right ventricular cardiomyopathy diagnosis? *World J Cardiol* 2014; 6(7): 675-681 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i7/675.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i7.675>

INTRODUCTION

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetic form of cardiomyopathy characterized by fibro-fatty replacement primarily of right ventricular muscle. ARVC is inherited predominantly as an autosomal dominant pattern. There are also recessive forms described caused by mutations in the plakoglobin and desmoplakin (*e.g.*, Naxos Disease, Carvajal Syndrome) which are associated with a cutaneous phenotype^[1-3]. Although the name describes a “right” ventricular process, it is now recognized that left ventricular involvement is much more common and acknowledged at an earlier stage than before^[4-6]. In the early stage of the disease, structural changes may be absent or subtle and are due to myocardial injury, inflammation and repair^[7] and are confined to a localized region of the right ventricle (RV), typically the RV outflow tract, pulmonary infundibulum or the RV apex, which form a “triangle of dysplasia”^[8] (Figure 1, see arrows). These areas are eventually replaced by fibrous and fatty tissue^[7,9] resulting in aneurysm formation, which is commonly seen in the basal inferior wall below the tricuspid valve^[10]. Ventricular aneurysms at these sites can be considered pathognomonic of ARVC^[7] (Figure 2, see arrows). These changes contribute to the electrical instability, which triggers ventricular tachycardia and sudden cardiac death (SCD)^[11,12].

Clinical manifestations of ARVC are variable. The most common symptoms are palpitations and syncope, due to the occurrence of ventricular tachycardia. Occasionally, SCD may be the first event. ARVC is a relatively common cause of unexpected SCD in the young, especially in athletes^[13,14]. Progressive structural involvement results in failure of the right or left ventricle depending on which one is predominantly affected and eventually to biventricular heart failure^[15-17]. Following the diagnosis of ARVC or the occurrence of SCD due to suspected ARVC; evaluation of family members is frequently initiated.

Histological confirmation is required for the definitive diagnosis of ARVC; however myocardial biopsy may not necessarily be sensitive due to the segmental nature of the disease^[18]. An International Task Force (Table 1) was developed in 1994, which proposed major and minor criteria for the diagnosis of ARVC^[19]. The diagnostic criteria depends on a variety of factors including functional

Table 1 Task force criteria for the diagnosis of arrhythmogenic right ventricular cardiomyopathy^[19]

1 Global and/or regional dysfunction and structural alterations (detected by echocardiography, angiography, magnetic resonance imaging, or radionuclide scintigraphy)
Major: Severe dilatation and reduction of right ventricular ejection fraction with no (or only mild) left ventricular impairment. Localized right ventricular aneurysms (akinetic or dyskinetic areas with diastolic bulging). Severe segmental dilatation of the right ventricle
Minor: Mild global right ventricular dilatation and/or ejection fraction reduction with normal left ventricle Mild segmental dilatation of the right ventricle Regional right ventricular hypokinesia
2 Tissue characterization of wall
Major: Fibro-fatty replacement of myocardium on endomyocardial biopsy
3 Repolarisation Abnormalities
Minor: Inverted T waves in right precordial leads (V2 and V3) in people aged > 12 yr, in absence of right bundle branch block
4 Depolarization/conduction abnormalities
Major: Epsilon waves or localized prolongation (> 110 ms) of the QRS complex in right precordial leads (V1-V3)
Minor: Late potentials (signal-averaged ECG)
5 Arrhythmias
Minor: Left bundle branch block type ventricular tachycardia (sustained and non-sustained) by ECG, Holter or exercise testing. Frequent ventricular extra-systoles (> 1000/24 h) by Holter
6 Family history
Major: Familial disease confirmed at necropsy or surgery
Minor: Family history of premature sudden death (< 35 yr) due to suspected right ventricular dysplasia. Familial history (clinical diagnosis based on present criteria)

The diagnosis of arrhythmogenic right ventricular cardiomyopathy (ARVC) would be fulfilled in the presence of 2 major criteria or 1 major plus 2 minor or 4 minor criteria from the different groups. ECG: Electrocardiogram.

and structural abnormalities on imaging such as cMR or echocardiography, tissue characterisation (*i.e.*, endomyocardial biopsy), repolarization and depolarisation abnormalities in the electrocardiogram (ECG), arrhythmias, family history and genetic analysis^[20]. These criteria although were highly specific, lacked the sensitivity for early disease^[21,22]. In 2010, revised criteria were proposed to include quantitative parameters particularly for the imaging studies (Table 2) to improve diagnostic sensitivity whilst maintaining specificity^[5].

cMR has an important role in the diagnosis of ARVC as it allows three-dimensional visualisation of the ventricles and is very useful in the assessment of functional and structural abnormalities^[23]. Previous studies have demonstrated that cMR has high sensitivity and specificity for ARVC diagnosis^[24,25] and also play an important role in the evaluation of ARVC even in patients who do not meet the Task Force Criteria^[26]. Due to the high sensitivity and specificity, cMR has also been suggested as a routine method of examination if ARVC is clinically suspected^[25]. Apart from excluding ARVC, cMR is also sometimes useful in finding ARVC mimics or other clinically significant findings^[26,27].

The requests to rule out ARVC constitute a significant proportion of the total cMR referrals in our institution from the cardiologists. Although the previous studies

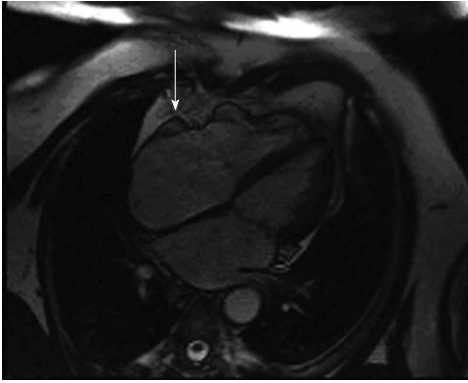


Figure 1 Cardiac magnetic resonance 4 chamber image from cine imaging demonstrating dyskinesia of the right ventricle free wall.

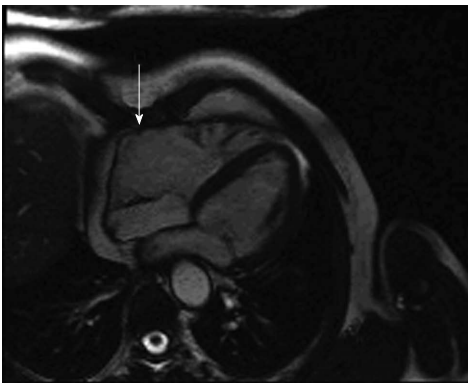


Figure 2 Cardiac magnetic resonance 4 chamber image from cine imaging demonstrating aneurysm of the basal segment free right ventricle wall.

have demonstrated the critical role of cMR in the diagnosis of ARVC, the impact of cMR outcome in an unselected population is not widely analysed. So this study was undertaken to find out what percentage of patients with a suspected diagnosis of ARVC referred for cMR had positive clinical results and whether the outcome helped in deciding further patients' care.

MATERIALS AND METHODS

Study population

All the patients who were referred for cMR with a suspected diagnosis of ARVC or with a family history of suspected or confirmed ARVC from May 2006 to February 2010 in our institution were included in this retrospective analysis (Table 3). A total of 121 patients were referred in this period. Seven patients were not scanned due to claustrophobia. Therefore, the study included 114 patients. Out of the 114 patients who underwent cMR, 63 patients were male (55%) and 51 were female (45%). The age range was from 16 to 83 years, however 82% of the patients were between 20 and 60 years of age. The majority of these patients (84%) were referred from the cardiologists in our teaching institution, and the rest came from the cardiologists from the five district general hospitals and one private hospital. The three most common reasons for referral were arrhythmias (30%), fam-

Table 2 Revised task force criteria for imaging^[5]

Major
By 2D echo:
Regional RV akinesia, dyskinesia or aneurysm and one of the following (end diastole):
1 Parasternal long axis view RVOT (PLAX) ≥ 32 mm (corrected for body size (PLAX/BSA) ≥ 19 mm/m ²)
2 Parasternal short axis view RVOT (PSAX) ≥ 36 mm (corrected for body size (PSAX/BSA) ≥ 21 mm/m ²)
3 Or fractional area change (FAC) $\leq 33\%$
By MRI:
Regional RV akinesia or dyskinesia or dyssynchronous RV contraction and one of the following:
1 Right ventricular end diastolic volume (RVEDV/BSA) ≥ 110 mL/m ² (male) or ≥ 100 mL/m ² (female)
2 Or RVEF $\leq 40\%$
By RV angiography:
Regional RV akinesia, dyskinesia or aneurysm
Minor
By 2D echo
Regional RV akinesia or dyskinesia and one of the following (end diastole):
1 Parasternal long axis view RVOT (PLAX) ≥ 29 - < 32 mm (corrected for body size (PLAX/BSA) ≥ 16 - < 19 mm/m ²)
2 Parasternal short axis view RVOT (PSAX) ≥ 32 - < 36 mm (corrected for body size (PSAX/BSA) ≥ 18 - < 21 mm/m ²)
3 Or FAC $> 33\%$ - $\leq 40\%$
By MRI
Regional RV akinesia or dyskinesia or dyssynchronous RV contraction and one of the following:
1 Right ventricular end diastolic volume/BSA ≥ 100 - < 110 mL/m ² (male) or ≥ 90 - < 100 mL/m ² (female)
2 RVEF $> 40\%$ - $\leq 45\%$

RVEF: Right ventricular ejection fraction; RVOT: Right ventricular outflow tract; FAC: Fractional area change.

ily history of sudden death (20%) and abnormal RV on echocardiography or on a previous cMR (19%). The other reasons for referral included history of palpitations and syncope, Supra-ventricular tachycardia, suspected Brugada syndrome, abnormal left ventricle (LV) and dilated cardiomyopathy on echo, frequent ectopics and cardiac arrest.

The institutional review board approved this retrospective study. Since this was a service evaluation, formal ethical approval was not required. However, the patients' confidentiality was respected.

Acquisition protocol

All the cardiac magnetic resonance imaging (MRI) scans were performed on a Siemens Avanto 1.5 Tesla magnetic resonance scanner using a body coil. The ARVC protocol included: (1) scout images; (2) dark blood Half-Fourier Acquisition Single-Shot Turbo Spin-Echo imaging in 3 planes-axial, coronal and sagittal; and (3) a series of balanced, steady state free precession (25 phase) cine images, with standard views in the following planes; Vertical long-axis (VLA) or 2-chamber; 4-chamber (FCH); Left ventricular outflow tract (LVOT) or 3 chamber; Right ventricular outflow tract; short axis stack at 10 mm intervals to allow quantification of ventricular volumes and function; Axial stack to assess for RV free wall regional wall motion abnormality; (4) gadolinium was adminis-

Table 3 Study population

Time period	May 2006-Feb 2010
Total No. of patients referred	121
Total No. of patients scanned	114
Age range	16 to 83
16-20	7 (6%)
21-30	19 (17%)
31-40	25 (22%)
41-50	28 (24%)
51-60	21 (18%)
61-70	10 (9%)
71-80	3 (3%)
81-90	1 (1%)
Males	63 (55%)
Females	51 (45%)
Referrals	
Teaching Hospitals	96 (84%)
District General Hospitals	18 (16%)
Reasons for referral	
Arrhythmias	34 (30%)
Family history of sudden death	23 (20%)
Others	57 (50%)
Others	
Abnormal RV in echo	22 (19%)
Brugada syndrome	8 (7%)
Syncope	8 (7%)
Palpitations	6 (5%)
Dilated cardiomyopathy in echo	4 (3.5%)
Frequent ectopics	4 (3.5%)
SVT	3 (3%)
Abnormal LV	1 (1%)
Cardiac arrest	1 (1%)

RV: Right ventricle; LV: Left ventricle.

tered at a rate of 0.1 mmol/kg and followed by early gadolinium inversion recovery images in the LVOT, FCH, VLA planes, with an inversion time (TI) of 440 ms; and then (5) late gadolinium inversion recovery images were obtained of the LVOT, FCH and VLA planes and as a short axis stack; repeated with a phase swap, with the TI set to produce optimal myocardial nulling. Right ventricular volumes were analysed from the short axis stack and compared with indexed normal values.

Data analysis

Patient details including age, sex, ECG and echo abnormalities, family history, MRI findings and information about clinical management were obtained. We analyzed the data for cMR findings, which fitted with the major and minor criteria for ARVC. The major MRI criteria for ARVC included severe global/segmental dilatation of the RV and global systolic dysfunction. The minor criteria included mild global/segmental dilatation of the RV, regional contraction abnormalities and global diastolic dysfunction according to the Task Force Criteria. The gadolinium enhancement if seen was also recorded. At least 2 radiologists and 1 cardiologist jointly reported the scans. The MRI findings were correlated with clinical outcome.

Statistical analysis

The overall sensitivity and specificity of cMR to diagnose

Table 4 Results

RV abnormalities related to ARVC	19 (17%)
Major	4 (4%)
Minor	13 (11%)
Non specific	2 (2%)
Other diagnoses	95 (83%)
Normal	63 (55%)
Dilated cardiomyopathy	8 (7%)
Left to right shunt	1 (1%)
RV infarction	1 (1%)
LV infarction	1 (1%)
Other mild abnormalities	21 (18%)
Clinically proven ARVC	5 (4%)
cMR major	3
cMR minor	2
Non specific (out of 2)	0
Others (out of 95) ¹	0
Family history of sudden death	23
Clinically proven ARVC	1
Minor and non specific criteria for ARVC (not clinically proven)	3
Normal	15
LV hypertrophy	1
LV dyssynchrony	1
LV infarct	1
Dilated cardiomyopathy	1

¹Clinical data was available for only 81 patients. RV: Right ventricle; ARVC: Arrhythmogenic right ventricular cardiomyopathy; LV: Left ventricular.

ARVC and the positive and negative predictive values in three different groups (arrhythmias, family history and others) were calculated. Values were expressed in percentages.

RESULTS

CMR findings with abnormalities related to the right ventricle were classified as major, minor and non-specific according to whether they met major or minor cMR diagnostic criteria of ARVC. There were also some non-specific findings related to the RV such as mild dyskinesia, thinning of RV wall and mildly impaired RV function.

Of the 114 patients, 19 patients had RV related abnormalities (Table 4). Of these, 4 patients had major cMR criteria, 13 had minor criteria and 2 patients had non-specific features. Out of the 4 who had major criteria for ARVC, 3 were clinically proven to have ARVC of whom 2 had an implantable cardioverter defibrillator (ICD) fitted. The third patient, who also had family history of ARVC, died soon after the diagnosis. Only this patient (1 out of 4) showed extensive RV free wall late gadolinium enhancement. The fourth patient did not meet the full diagnostic criteria, but still had an ICD fitted for recurrent episodes of ventricular tachycardia (VT). Out of the 13 patients with minor criteria, 2 were clinically proven to have ARVC. None of these patients had late gadolinium enhancement. Eight patients with either minor or non-specific criteria had repeat MR scan, which suggested either no significant or mild change compared to the earlier scans. Out of the 95 patients who had nega-

Table 5 Positive predictive value in different groups

Clinical history - No of patients	Major criteria present	Clinically proven ARVC	Positive predictive value	Minor criteria present	Clinically proven ARVC	Positive predictive value
Arrhythmia (30%)	2	1	50%	3	2	67%
Family history of SCD (20%)	1	1	100%	1	0	0%
Others (50%)	1	1	100%	9	0	0%

ARVC: Arrhythmogenic right ventricular cardiomyopathy; SCD: Sudden cardiac death.

tive cMR for ARVC, clinical data were available for 81 patients and none of them had a positive clinical diagnosis of ARVC. Of these patients, 63 patients had normal scans and 8 had dilated cardiomyopathy. Other significant diagnoses included left to right shunt (1), LV infarction (1) and RV infarction (1). The remaining patients had mild chamber or aortic root abnormality.

There were 23 patients referred with a family history of sudden death. One had major cMR criteria, one had minor cMR criteria and 2 had non-specific findings related to the RV on cMR. Apart from the one patient who had major criteria, there was no other clinically proven ARVC in patients with family history. Out of the remaining 19 patients, 15 patients had normal scans, 1 had LV hypertrophy, 1 had LV dyssynchrony, 1 had an LV infarct with RV impairment and one had dilated cardiomyopathy.

In summary, of the total cohort of 114 patients, 17% had scans showing abnormalities related to RV and 83% had scans not suggestive of ARVC. Four percent of the study population had clinical proven ARVC and in all of them, MR was positive for either major or minor criteria. Excluding the 14 patients with no clinical data and final diagnosis, the overall sensitivity of the test was 100%, specificity 87%, positive predictive value 29% and the negative predictive value 100%. If split by criteria, the positive predictive value for major criteria was 75% and minor criteria 15%. The positive predictive values for different patient groups (arrhythmias (30%), family history (20%), and others (50%)) are given in Table 5. The negative cMR diagnoses were reassuring in terms of clinical management especially for the patients with family history of sudden death.

DISCUSSION

This study has shown that in this cohort of patients 15% fulfill imaging criteria for ARVC, with a subsequent 75% of cases fulfilling Task Force criteria for the diagnosis of ARVC if they had a major CMR criterion. Conversely a negative CMR scan for ARVC on imaging criteria translated into no subsequent diagnosis of ARVC.

Referrals for cMR in our centre come from a variety of sources, including the Inherited Cardiac Conditions service and the Arrhythmia service, which are based regionally at our institution. In addition, referrals are received from general cardiology clinics in our institution and from the district general hospitals in our region. The scans are reported jointly by radiologists and cardiologists at multidisciplinary meetings to maximize the clinical rel-

evance of the reports.

In our study 15% of the patients fulfilled imaging criteria for ARVC. Major criteria were found in 4% of the cases and minor criteria were found in 11%. Data from other centers report a detection rate of 3%-10%^[26,27], for unselected cMR referrals for possible ARVC. Therefore our detection rate does not differ significantly from these. These detection rates may fall following implementation of the modified Task Force Criteria^[5], with one study showing a significant drop in the number of positive scans^[28].

Our study showed that none of the patients with CMR scans that were negative for ARVC on imaging criteria were subsequently diagnosed with ARVC. This is an important finding and is reassuring to the clinicians involved. This negative predictive value of 100% compares to previous studies which have also shown the diagnostic accuracy of CMR in the diagnosis of ARVC^[28]. While it may be perceived by some that many studies proved to be negative, we claim that these studies are particularly helpful to the clinician and the patient alike in excluding important pathology such as ARVC, particularly in patients presenting with arrhythmias or those with a family history of either ARVC or of sudden cardiac death. Interestingly we also found evidence of other pathologies in 11% of patients with scans negative for ARVC on imaging criteria, the majority of these were diagnosed as dilated cardiomyopathy. This is in keeping with other studies, which report an incidence of significant other etiologies being diagnosed in 4%-8% of the cases^[26,27].

We have also shown that scans positive for ARVC on imaging criteria translate into a high percentage of patients formally fulfilling Task Force criteria for ARVC (75% of cases with a major imaging criteria, 15% with a minor criteria) and that no scans negative for ARVC on imaging criteria were subsequently diagnosed with ARVC. These reflect a reassuring performance by our clinically effective service where scans are reviewed in a joint cardiology and radiology multidisciplinary team meeting. In conclusion, CMR is a useful tool for excluding ARVC, because of a high negative predictive value and is especially helpful in patients with family history of sudden death. A positive scan correlates well with clinical findings. The technique is cost effective as the positive or negative outcomes have significant impact on the clinical decision-making.

Limitations of this paper are that the number of patients studied were small, with no control group and this precluded more detailed statistical analysis, such as hazard

ratio's. Further work in this area should include a larger study population.

COMMENTS

Background

Arrhythmogenic right ventricular cardiomyopathy (ARVC), an inherited disorder is a relatively common cause of sudden death especially in young athletes. The diagnostic criteria is however not simple and depends on fulfilling modified Task Force Criteria which involves a lot of diagnostic work up including multimodality imaging. Cardiac magnetic resonance imaging (cMR) is one such useful tool and also the one frequently requested to evaluate for ARVC. Although the previous studies have demonstrated the critical role of cMR in the diagnosis of ARVC, the impact of cMR outcome in an unselected population is not widely analysed.

Research frontiers

The definitive diagnosis requires endomyocardial biopsy, which is an invasive procedure, however even the biopsy may still not be sensitive due to the patchy nature of the disease. Cardiac MR has emerged over the years as a very useful non-invasive modality of choice and the research hotspot is to find out whether it can serve as a one-stop shop for the evaluation of ARVC.

Innovations and breakthroughs

Data from other centers report a detection rate of 3%-10%, for unselected CMR referrals for possible ARVC. This study results show 4% of the referrals had positive clinical diagnosis and none of the patients with negative CMR scans were subsequently diagnosed with ARVC. This is an important finding and is reassuring to the clinicians involved. Although significant proportion of studies proved to be negative, the authors claim that these studies are particularly helpful to the clinician and the patient alike in excluding important pathology such as ARVC, particularly in patients presenting with arrhythmias or those with a family history of either ARVC or of sudden cardiac death.

Applications

The study results show that cardiac MR is a useful tool for excluding ARVC, because of a high negative predictive value and the positive scan also correlates well with clinical findings, which makes the study cost effective as both the outcomes have significant impact on the clinical decision-making.

Terminology

"Arrhythmogenic cardiomyopathy" - "cardiomyopathy" refers to disease of the heart muscle which when affected by inflammation with subsequent fibrosis and fat infiltration can cause "arrhythmogenic" potential which triggers the heart muscle to produce very high heart rates such as ventricular tachycardia and fibrillation due to electrical instability which can result in sudden death.

Peer review

The manuscript is well written and highly organized. The readability is excellent. In this retrospective analysis of 114 patients referred for CMR because of arrhythmias or family history of sudden death, the results of CMR were classified depending on both functional and tissue characterisation and the clinical information were used. The assessment and judgment of the images of CMR was performed jointly by radiologist and cardiologist. This study shows that CMR has an important role in the diagnosis of ARVC as it allows 3-D visualization of the ventricles and CMR is sometimes useful in finding other disorders for patient's symptoms.

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Rare case of coronary to pulmonary vein fistula with coronary steal phenomenon

Emad A Barsoum, Faisal B Saiful, Deepak Asti, Rewais Morcus, Georges Khoeiry, James Lafferty, Donald A McCord

Emad A Barsoum, Faisal B Saiful, Rewais Morcus, Georges Khoeiry, James Lafferty, Donald A McCord, Department of Medicine, Staten Island University Hospital, New York, NY 10305, United States

Faisal B Saiful, Deepak Asti, Georges Khoeiry, James Lafferty, Donald A McCord, Division of Cardiology, Staten Island University Hospital, New York, NY 10305, United States

Author contributions: All authors contributed to this work.

Correspondence to: Emad A Barsoum, MD, Department of Medicine, Staten Island University Hospital, 475 Seaview Ave, Staten Island, New York, NY 10305,

United States. dr_barsoum@yahoo.com

Telephone: +1-347-6669321 Fax: +1-718-2268695

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Key words: Coronary artery fistula; Coronary artery anomalies in adult; Coronary artery disease

Core tip: This report highlights the presence of an extremely rare coronary anomaly in adult, in the form of a fistula between left anterior descending coronary artery and left superior pulmonary vein with steal phenomenon causing angina that resolved by medical treatment.

Barsoum EA, Saiful FB, Asti D, Morcus R, Khoeiry G, Lafferty J, McCord DA. Rare case of coronary to pulmonary vein fistula with coronary steal phenomenon. *World J Cardiol* 2014; 6(7): 682-684 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i7/682.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i7.682>

Abstract

Coronary artery fistulas are abnormal connections between coronary artery territories and cardiac chambers or major vessels, most of them are congenital. Patients with coronary artery fistula can be asymptomatic or present with different symptoms like angina. Cardiac computed tomography (CT) is one of the best modalities for diagnosis. We present an elderly patient that presented with angina symptoms, non invasive stress test was positive for ischemic heart disease, coronary angiogram could not reveal any obstructive lesions, but an abnormal branch of the left descending coronary artery (LAD), cardiac CT showed fistula that connect left anterior descending coronary artery to left superior pulmonary vein. Our case is extremely rare as most of the reported cases were fistulas between LAD and pulmonary artery, but in our case the fistula between LAD and left superior pulmonary vein. In addition, our patients' symptoms resolved with anti-ischemic medical treatment without any surgical intervention.

INTRODUCTION

Coronary artery anomalies are found in 1% of coronary angiograms^[1]. Some of these anomalies are clinically insignificant but, many others are associated with serious morbidity and potential mortality. Coronary artery anomalies can be detected by a variety of means including echocardiography, coronary artery angiography and multidetector-row computed tomography^[2,3].

The following case report describes an elderly patient presenting with angina, whose coronary angiography and cardiac computed tomography (CT) revealed an abnormal communication between the left anterior descending (LAD) and the left superior pulmonary vein.

CASE REPORT

A 67-year-old man presented to the office with exertional chest pain of six weeks. He had a past medical history significant for hypercholesterolemia and gastro esopha-

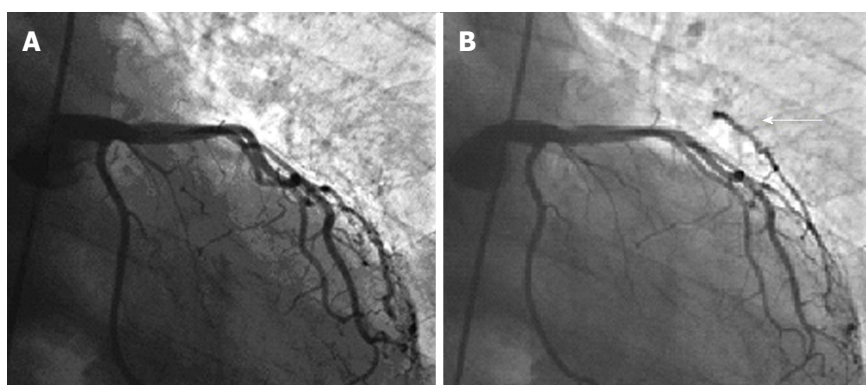


Figure 1 Coronary angiogram. A: Showing coronaries without significant atherosclerotic lesion; B: Showing fistula arises from left anterior descending.

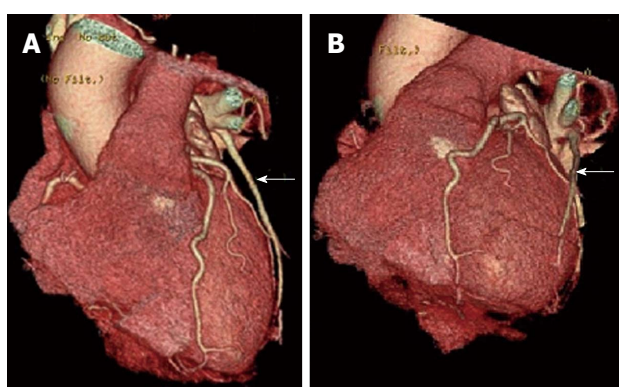


Figure 2 Multislice computed tomographic angiography. A: Showing fistula between left anterior descending (LAD) and superior pulmonary vein; B: Showing fistula between LAD and superior pulmonary vein.

geal reflux disease. He quit smoking 30 years ago. A thallium stress test revealed a moderate sized, completely reversible, anterior and anterolateral wall defect suggestive of LAD territory ischemia. Coronary angiography was performed, which failed to reveal any obstructive disease in the LAD as well any other coronary vessel. However, in the distal segment of the LAD there was a small aneurysmal dilatation and a communication with an extracardiac vessel that was well opacified with antegrade injection of contrast (Figure 1). We concluded that it was likely to be a fistula between the LAD and a segment of the left pulmonary artery system. We decided to get a CT angiogram of the chest to better delineate the nature of the fistula as an outpatient. The patient was discharged home on a regimen including a beta-blocker, statin, ACE inhibitor and a long acting nitrate. Soon after, a multislice computed tomographic angiography of the thorax and coronaries was performed. A fistula arising from the distal LAD and connecting to the left superior pulmonary vein was elucidated (Figure 2). The patient has been followed up at one and three month intervals. He continues to do very well with optimal medical therapy and remains free of exertion and rest angina.

DISCUSSION

Coronary artery fistula (CAF) is an abnormal connection between coronary artery territories and cardiac chambers

or major vessels, CAF represent 17% of angiographic diagnosed anomalies^[1]. Majority of them are congenital, but can be acquired secondary to increasing application of intravascular diagnostic instrumentations and therapeutic procedures or even secondary to blunt or penetrating trauma^[4-11].

Patient with coronary artery fistulas usually asymptomatic that the fistula accidentally detected by echocardiography and coronary angiography, but patients may have varied symptoms such as angina pectoris, palpitations, syncope, congestive heart failure, and may even present with sudden cardiac death. In some cases, physical examination may or reveal a murmur if the flow is significant^[12].

The majority of reported LAD fistulas have been between the LAD and pulmonary artery, but in our case the anomaly is between the LAD and the left superior pulmonary vein. Also, it is interesting that we have concomitant coronary steal phenomenon by the pulmonary venous system. This is evident due to the presence of angina symptoms and a reversible defect on nuclear imaging that is not explained by coronary artery disease. It is plausible that a small fistulas increase in size with advancing age secondary to changes in vessels compliance and pressure and as it reaches a certain flow threshold, begins to exhibit steal phenomenon.

The main treatment of symptomatic coronary fistulas is surgical and a variety of operative techniques have been described in the literature including internal closure of the fistula from within the distal communication, distal ligation alone, proximal and distal ligations and closure from within the aneurysmal coronary artery^[13,14]. In addition, transcatheter retrograde coil embolization became a safe and effective alternative to standard surgical closure^[15,16]. However, our patient's symptoms are resolved with optimal anti ischemic medical therapy.

In a conclusion, Coronary artery fistula to pulmonary vein is extremely rare, medical treatment is effective to resolve patient's symptoms, long term follow up is highly recommended.

COMMENTS

Case characteristics

A 67-year-old man with a history of hypercholesterolemia and gastro esopha-

geal reflux disease presented with exertional chest pain.

Clinical diagnosis

Normal physical exam.

Differential diagnosis

Ischemic heart disease, non cardiac causes of chest pain.

Laboratory diagnosis

Cardiac enzymes were within normal limits.

Imaging diagnosis

Coronary angiography and cardiac computed tomography revealed an abnormal communication between the left anterior descending (LAD) and the left superior pulmonary vein.

Treatment

The patient was treated with beta-blocker, statin, ACE inhibitor and a long acting nitrate.

Related reports

A fistula between left anterior descending coronary artery and left superior pulmonary vein is extremely rare. Most of the reported cases are between a coronary artery and the pulmonary artery.

Term explanation

Coronary artery anomalies are rare in adults, but they can induce angina symptoms.

Experiences and lessons

This case report represents rare fistula between LAD and left superior pulmonary vein, cardiac computed tomography scan was a sensitive modality in the detection of the fistula and the patient improved on medical treatment without surgical intervention.

Peer review

This article presents an extremely rare fistula between LAD and left superior pulmonary vein in adult.

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Worsening of coronary spasm during the perioperative period: A case report

Hiroki Teragawa, Kenji Nishioka, Yuichi Fujii, Naomi Idei, Takaki Hata, Shuji Kurushima, Tomoki Shokawa, Yasuki Kihara

Hiroki Teragawa, Kenji Nishioka, Yuichi Fujii, Naomi Idei, Takaki Hata, Shuji Kurushima, Tomoki Shokawa, Yasuki Kihara, Department of Cardiovascular Medicine, Hiroshima University Hospital, Hiroshima 734-8551, Japan

Author contributions: Teragawa H wrote the manuscript; Nishioka K, Fujii Y, Idei N, Hata T, Kurushima S and Shokawa T collected data; Kihara Y evaluated the study and revised the manuscript.

Correspondence to: Hiroki Teragawa, MD, PhD, Department of Cardiovascular Medicine, Hiroshima General Hospital of the West Japan Railway Company, 3-1-36 Futabanosato, Higashi-ku, Hiroshima 734-8551, Japan. hiroteraga71@gmail.com
Telephone: +81-82-2621171 Fax: +81-82-2621499

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Abstract

We present the case of a 65-year-old male with vasospastic angina (VSA) whose condition worsened during the perioperative period. He had been diagnosed with VSA 10 years prior. He was treated with two types of vasodilators and had not experienced any chest symptoms for 5 years. At this juncture, he underwent surgery for relapsed maxillary sublingual carcinoma. He had taken two vasodilators one day prior to surgery. Intravenous infusion of nitroglycerin (NTG) was initiated immediately before the surgery and continued the following day. Instead of stopping NTG, a dermal isosorbide dinitrate tape was applied on post-operative day 1. Two days later, a complete atrioventricular block with pulseless electrical activity appeared. After cardiopulmonary resuscitation, emergent coronary angiography showed severe coronary spasm in both the left and right coronary arteries. Intracoronary infusion of nitroglycerin and epinephrine with percutaneous cardiopulmonary support relieved the coronary spasm. During the perioperative period, several factors can trigger coronary vasospasm, including the discontinua-

tion of vasodilators. Thus, surgeons, anesthetists, and cardiologists should watch for coronary vasospasm during this period and for worsening coronary spasm when discontinuing vasodilators in patients at risk for VSA.

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Key words: Coronary vasospasm; Perioperative period; Discontinuation of vasodilator

Core tip: Coronary spasm during the perioperative period often emerges severely as either cardiogenic shock or ventricular fibrillation. Although there are several surgery-related factors that influence the activity of coronary spasm, discontinuing vasodilators during the perioperative period is an important problem in patients with vasospastic angina (VSA). We encountered an out-patient with VSA whose condition had been stabilized using two types of vasodilators but subsequently worsened, leading to cardiogenic shock during the perioperative period. In light of this event, physicians should carefully evaluate their patients regarding the possibility of a coronary spasm during the perioperative period.

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INTRODUCTION

The abrupt cessation of vasodilators causes rebound coronary spasm in patients with vasospastic angina (VSA)^[1-6]. During the perioperative period, physicians sometimes have to discontinue oral vasodilators, even in patients with VSA. Furthermore, other factors that worsen VSA

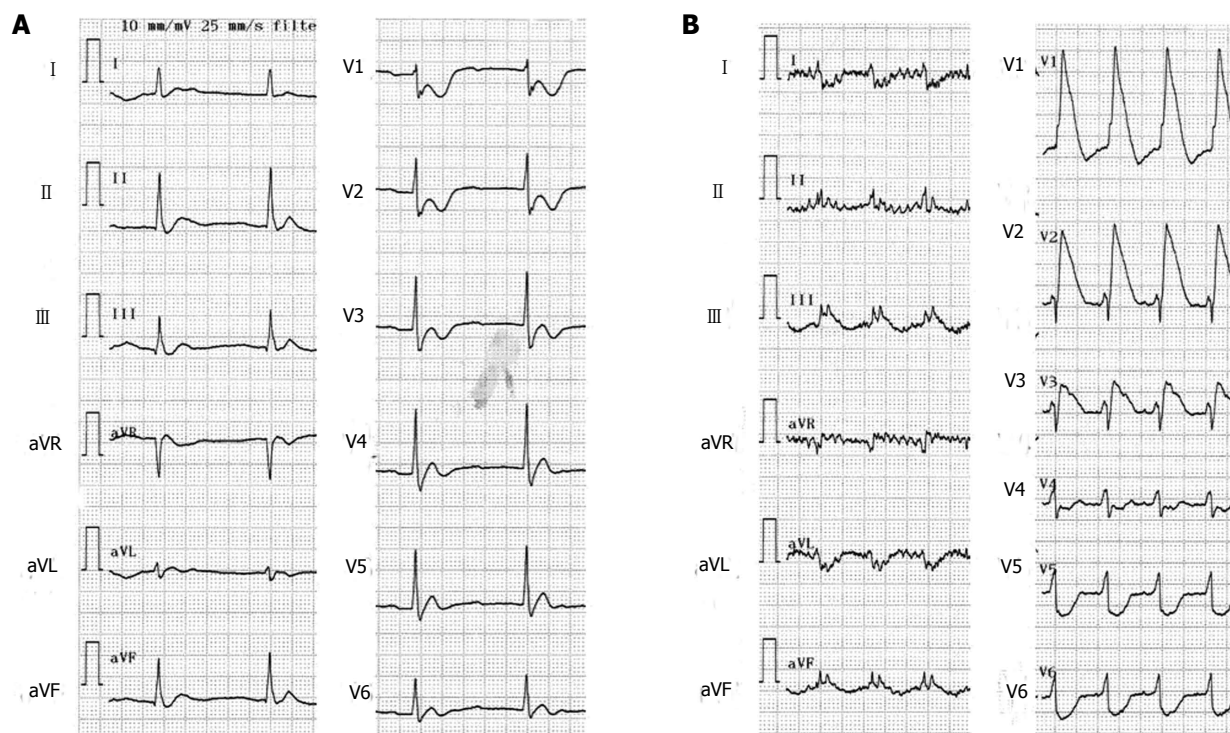


Figure 1 Electrocardiograms showed complete atrioventricular block when blood pressure decreased (A) and ST elevations were noted in leads II, III, aVF, and V1-3 immediately after cardiopulmonary resuscitation (B).

occur during the perioperative period. Thus, VSA activity may be accelerated during this interval. However, little is known about VSA management during this period^[7,8]. In this study, we present a case in which coronary spasm worsened during the perioperative period.

CASE REPORT

A 65-year-old male, who was diagnosed with VSA 10 years prior and treated with two types of vasodilators (benidipine hydrochloride, 8 mg/d, and nicorandil, 5 mg/d) was admitted to the Department of Otolaryngology at our institution to undergo surgery for relapsed maxillary sublingual carcinoma. He had a coronary risk factor due to smoking (30/d \times 37 years). With regard to the results of the interview and medical records, the patient experienced spontaneous and/or imminent episodes of VSA several times per year for the first 5 years, but he had experienced no chest symptoms for the past 5 years. On admission, his vitals were stable. Blood examination, electrocardiogram (ECG), and echocardiography showed no specific findings.

On pre-operative day 1, the patient took the same 2 vasodilators; this oral medication was stopped on the day of surgery. Instead, from the morning of the surgery, an intravenous infusion of nitroglycerin (NTG) at 2 mL/h was started. A large left maxillary resection and submaxillectomy were performed. On post-operative day 1, the patient was sedated with midazolam. Intravenous NTG was stopped on post-operative day 1, and a dermal isosorbide dinitrate tape was applied. In the evening of post-operative day 2, his blood pressure decreased, and ECG

showed a complete atrioventricular block (Figure 1A). Pulseless electrical activity (PEA) was subsequently noticed, and cardiopulmonary resuscitation was initiated with repeated infusions of epinephrine. Ventricular fibrillation and PEA were repeated and percutaneous cardiopulmonary support (PCPS) was started 1 h later. ECG at that time showed ST elevations in leads II, III, aVF, and V1-3 (Figure 1B). Emergent coronary angiography revealed an occlusion at the proximal segment of the right coronary artery, and severe and diffuse narrowing due to coronary spasms of the left coronary artery (Figure 2A). Intracoronary infusions of nitroglycerin and epinephrine (20-100 μ g) were repeated, which relieved the bilateral coronary spasms (Figure 2B). Intravenous NTG and dopamine were continued, and PCPS was removed 4 d later. From post-operative day 19, oral benidipine hydrochloride at 8 mg/d was started, in addition to intravenous NTG infusion. From post-operative day 21, NTG infusion was discontinued, and only oral benidipine hydrochloride was prescribed. Thereafter, the patient had no chest symptoms; however, he died on post-operative day 74 from hydrocephalus due to the original disease.

DISCUSSION

We describe a case with VSA, where the condition was stable for 5 years but worsened during the perioperative period. The cessation or reduction of vasodilators worsens coronary spasm in patients with VSA^[1-6]. In fact, we have seen several VSA cases in which noncompliance to vasodilators increased angina attacks in the clinical setting. In the perioperative period, vasodilators sometimes

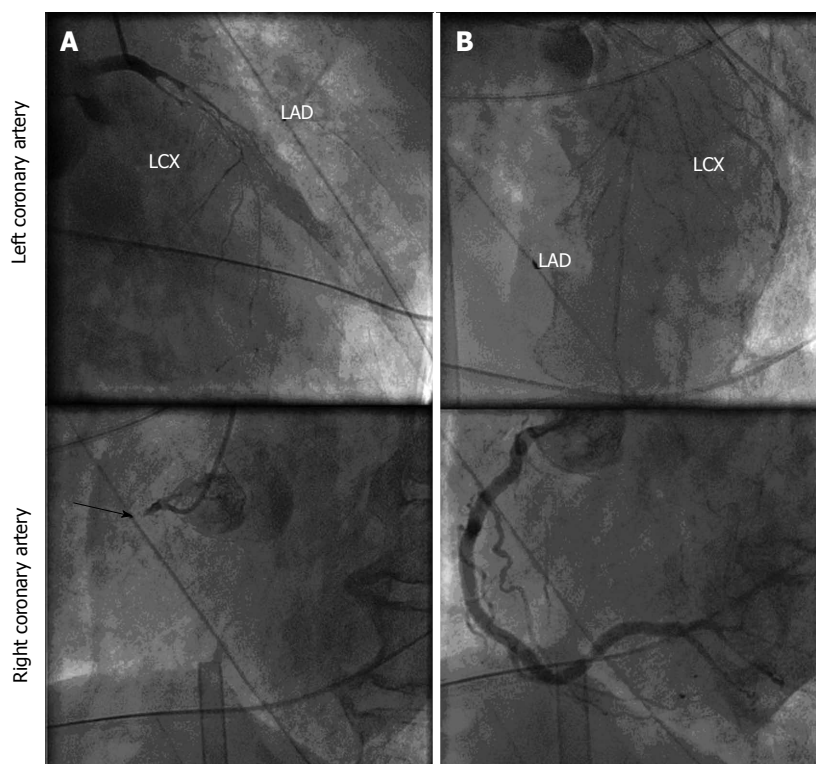


Figure 2 Emergent coronary angiography showed severe narrowing of the left anterior descending coronary artery and the left circumflex coronary artery and occlusion at the proximal segment of the right coronary artery (indicated by an arrow) in initial shots (A), and the trunks of the left anterior descending coronary artery, left circumflex coronary artery, and right coronary artery were found to be dilated despite remaining coronary spasm at the branches after intracoronary infusions of nitroglycerin and epinephrine (B). LAD: Left anterior descending coronary artery; LCX: Left circumflex coronary artery.

have to be stopped or reduced, even in patients with VSA. Thus, VSA management during the perioperative period is of pivotal importance, based on the knowledge gained from the present case.

Although the occurrence or exacerbation of VSA during the perioperative period is not frequent^[8], the problem certainly exists. At our institution, the incidence from 1999 to 2008 was 0.042% (17 cases/40466 operations, unpublished data). Koshiba *et al*^[7] reviewed the clinical characteristics of perioperative coronary spasm and raised several important points, including possible contributing factors and the surgical site. Contributing factors for perioperative coronary spasm may include inadequate anesthesia, use of vasopressors, vagal nerve stimulation, drugs other than vasopressors, epidural block, hypotension, mechanical stimulation of the heart, allergic reaction, and/or mental stress. Regarding the surgical site, abdominal surgery was performed in 49% of cases. At our institution, abdominal surgeries comprised 76% of all perioperative VSA cases (13 of 17), and upper abdominal surgery was the most frequent type with 65% (11 of 17 cases, unpublished data). An increase in vagal nerve stimulation, a possible contributing factor, may be the result of abdominal surgery, which was the most frequently performed procedure. Nagayoshi *et al*^[8] reported a case of perioperative coronary spasm at their institution and showed that the surgical risks are fairly low in patients who developed coronary spasm in the perioperative period. Preoperative consultations with a cardiologist were reported in only 2 of 18 cases with perioperative coronary spasm. Therefore, a cardiologist should be consulted before surgery in patients with known VSA or coronary artery disease (CAD). In the clinical setting,

even though the presence of VSA or CAD has not been indicated, we sometimes encounter patients who have taken vasodilators and experienced no chest symptoms. A preoperative cardiology consultation should be recommended, even for such patients. Cardiologists should check all patient information, including interview notes, medical records, and preoperative cardiovascular examinations, and consider the possibility of a coronary spasm. Discontinuing vasodilators, including calcium-channel blockers, during the perioperative period is another important factor^[1,9]. In principle, vasodilators should be taken during the perioperative period; however, the surgery, which normally requires the discontinuation of these medications, must proceed as planned. In the present case, where a large left maxillary resection and submaxillectomy were performed, vasodilators were discontinued during the surgery. Under such circumstances, two alternatives exist: intravenous vasodilators and dermal nitrate tape/patch. We previously conducted a survey regarding the perioperative management of VSA patients by 31 cardiologists from the Hiroshima Prefecture (unpublished data). Based on the results of the questionnaires, intravenous vasodilators was not routine but frequently given, depending on the patient's VSA status, in 93% of cases. The patient's VSA status includes VSA episodes while taking oral medications, spasm-provocation test results (*e.g.*, organic stenosis, multivessel spasm, severely provoked spasm), and the number of vasodilators (more than or equal to 2). Intravenous vasodilators generally include NTG, nicorandil, and diltiazem, and determining when to stop the intravenous vasodilators may be a problem. They can be instantly stopped in VSA patients who can take oral vasodilators, particularly those with low

VSA activity. However, in patients who cannot take vasodilators orally, are compelled to use a dermal nitrate tape/patch, or have a high VSA activity, the use of intravenous vasodilators and oral medications (or dermal nitrate tape/patch) simultaneously may be necessary for 1 or 2 d. In the present case, because the patient used a dermal NTG tape after terminating intravenous NTG, the vasodilating effect may have decreased, leading to severe VSA. In contrast, a dermal nitrate tape/patch is usually prescribed for VSA patients with less active disease. In our survey, 69% of all effective answers indicated that the dermal nitrate tape/patch was used in patients who had taken vasodilators for an extensive period, in spite of a low probability of having VSA. Long-term use of nitrates, particularly a dermal nitrate tape/patch, increases the possibility of nitrate tolerance^[9-11]; therefore, physicians should be extremely judicious in prescribing these drugs.

Learning from the present case, we recommend the following management of coronary spasm during the perioperative period: (1) not only the cardiologist but also the surgeon and anesthetist have to rule out the presence of VSA according to the patient's medical records, interview, and results from the preoperative cardiovascular examinations; (2) if VSA is established, vasodilators must be included in the patient's treatment plan. If there is a suspicion of VSA, *i.e.*, the patient has been taking vasodilators for a long time, the regimen should be continued during the perioperative period; and (3) the use of intravenous vasodilators should be determined on the basis of the patient's VSA activity. During the transition from IV to oral administration, no gap should be allowed. The simultaneous use of two drugs may be required for 1 or 2 d.

In conclusion, perioperative coronary spasm has been proven to exist, despite its low frequency. In evaluating a patient's medical history and presenting symptoms, physicians should consider and investigate the possibility of perioperative coronary spasm. Intravenous infusion of vasodilators during the perioperative period is sometimes required, but a discontinuation may pose a risk of coronary spasm for the patient.

COMMENTS

Case characteristics

A 65-year-old male with vasospastic angina (VSA) whose condition worsened during the perioperative period.

Differential diagnosis

Authors' present a case in which coronary spasm worsened during the perioperative period.

Treatment

The patient had been diagnosed with VSA 10 years prior.

Peer review

Teragawa *et al* present an interesting case report about worsening of coronary spasm during the perioperative period in a patients with previous diagnosis of vasospastic angina.

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3D-echo in preoperative assessment of aortic cusps effective height

Jan Nijs, Sandro Gelsomino, Bastian BLJH Kietselaer, Orlando Parise, Fabiana Lucà, Jos G Maessen, Mark La Meir

Jan Nijs, Bastian BLJH Kietselaer, Orlando Parise, Mark La Meir, Cardiothoracic Surgery, University Hospital, 1020 Brussels, Belgium

Sandro Gelsomino, Fabiana Lucà, Jos G Maessen, Cardiothoracic Department, Maastricht University Hospital, 6229 HX Maastricht, The Netherlands

Author contributions: Nijs J and Gelsomino S conceived and drafted the article; Kietselaer BBLJH, Parise O and Lucà F contributed to the imaging and revised the manuscript for important intellectual contents; Maessen JG and La Meir M approved the final version of the manuscript to be published.

Correspondence to: Sandro Gelsomino, MD, PhD, Cardiothoracic Department, Maastricht University Hospital, P. Debye-laan 25, 6229 HX Maastricht, The Netherlands. sandro.gelsomino@libero.it

Telephone: +31-43-38881066

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Core tip: 3D transesophageal echocardiography and multiple plane reconstruction lets visualize all the three coaptation planes between the aortic valve (AV) cusps and overcomes the limits of 2-D echocardiography which allows to see only two of three AV coaptation planes and this may lead to misunderstanding of the underlying pathophysiological mechanism for aortic regurgitation and hence in unsuccessful repair. It is highly recommendable before AV repair to accurately study the complex three dimensional cusps anatomy and their geometric interrelation with aortic root.

Nijs J, Gelsomino S, Kietselaer BBLJH, Parise O, Lucà F, Maessen JG, La Meir M. 3D-echo in preoperative assessment of aortic cusps effective height. *World J Cardiol* 2014; 6(7): 689-691 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i7/689.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i7.689>

Abstract

Effective height, which represents the height difference between the central free margins and the aortic insertion lines can be easily determined by 2-D echocardiography and allows for identification of prolapse in the native cusps and assessment of prolapse correction after valve repair. Nonetheless, it allows to see only two of three aortic valve (AV) coaptation planes and this may lead to misunderstanding of the underlying pathophysiological mechanism for aortic regurgitation and hence in unsuccessful repair. In contrast, 3D transoesophageal echocardiography and multiple plane reconstruction lets visualize all the three coaptation planes between the AV cusps and it represents an invaluable tool in the assessment of aortic valve geometry. It is highly recommendable before AV repair to accurately study the complex three dimensional cusps anatomy and their geometric interrelation with aortic root.

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TO THE EDITOR

In the recent years aortic valve (AV) repair has gained increasing interest in the treatment of aortic root pathology^[1] as a feasible alternative to aortic valve replacement^[2].

Good results have been achieved with valve-preserving aortic replacement for patients in whom aortic regurgitation is solely caused by aortic root dilatation with morphologically preserved valve leaflets^[3]. In contrast, cusp repair still remains a surgical challenge when prolapse of cusp tissue impairs coaptation^[4].

The most prominent echocardiographic phenomenon indicating cusps prolapse is a decreased effective height (eH) which represents the height difference between the central free margins and the aortic insertion lines^[4]. This measurement, which depends on the complex re-

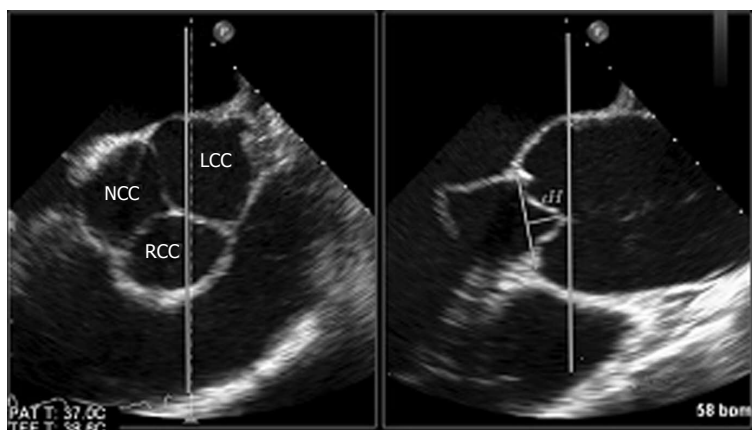


Figure 1 Established method of measuring the effective height in the 2-D short axis (left) and long axis image of the proximal aorta (right). As shown, only the coaptation between the right coronary cusp anteriorly and the left coronary cusp posteriorly can be measured. RCC: Right coronary cusp; LCC: Left coronary cusp; NCC: Noncoronary cusp.

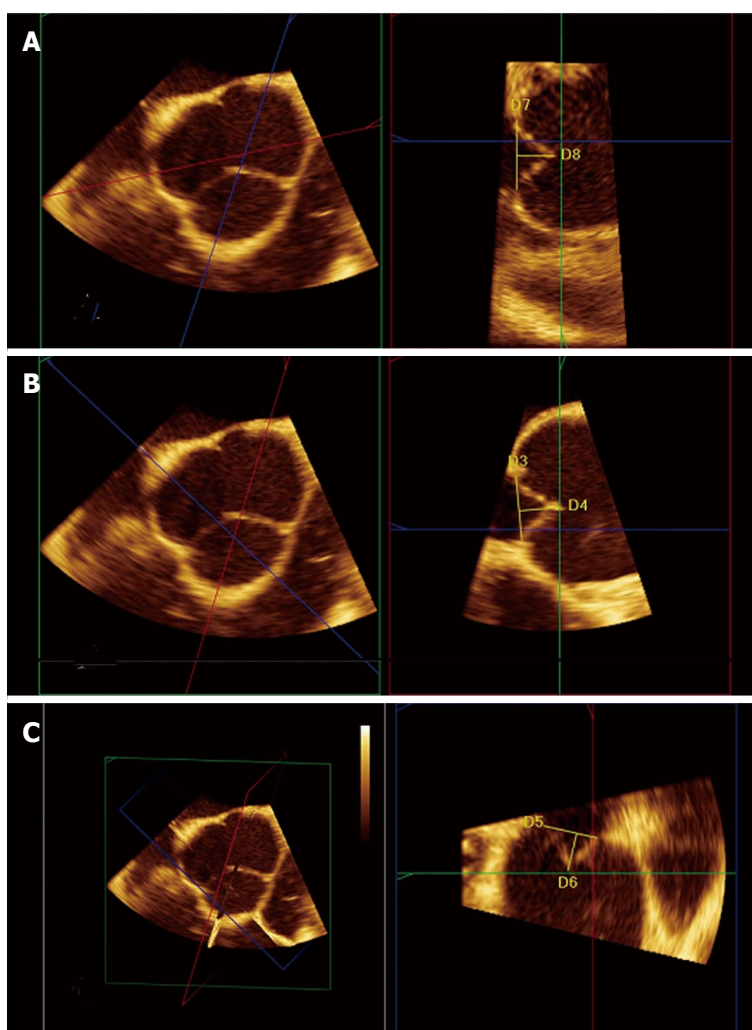


Figure 2 Images of a normal aortic valve from multiple plane reconstruction of the aortic valve with 3D transesophageal echocardiography. A: The red plane intersects the coaptation surface of the noncoronary cusp (NCC) and left coronary cusps (LCC). The yellow line labelled D8 (12.7 mm) represents the LCC effective height; B: The red plane intersects the coaptation surface of the LCC and right coronary cusps (RCC). The yellow line labelled D4 (13.9 mm) represents the effective height; C: The red plane intersects the coaptation surface of the NCC and RCC. The yellow line labelled D6 (12.7 mm) represents the effective height.

relationship of root and cusp, can be easily determined by 2-D echocardiography and allows for identification of prolapse in the native cusps and assessment of prolapse correction after valve repair. Nonetheless, with 2-D transesophageal echocardiography (2D-TEE) only two of three AV coaptation planes can be seen and the eH, a unidimensional value, can be measured only between the right coronary cusp anteriorly and either the non- or left coronary cusp (depending on probe rotation) posteriorly (Figure 1).

As a result, pathology of the AV cusp not included in the view may go undetected and this may eventually result in misunderstanding of the underlying pathophysiological mechanism for aortic regurgitation and hence in unsuccessful repair.

Recent development of real-time 3D transesophageal echocardiography (3D-TEE) allows multiple plane reconstruction (MPR) which lets visualize all the three coaptation planes between the AV cusps. Using MPR is possible to adjust the orthogonal imaging planes for op-

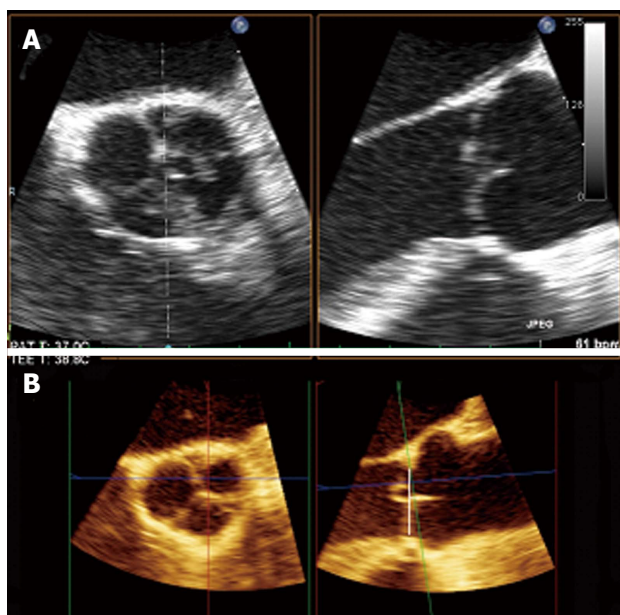


Figure 3 Patient male, 53-year-old. A: On 2 D there is no clear demonstration of prolapse; B: Multiple plane reconstruction of the aortic valve with 3D transesophageal echocardiography: on reformatted image a prolapse of left coronary cusps is shown (right).

timal visualization of all three aortic coaptation lines. By moving the planes is possible to identify the points where the cusps come together and to obtain, for each one, an accurate determination of the eH (Figure 2). 3D-echo multi-plane reconstruction is a valuable tool in the assessment of valve geometry and it is highly recommendable before AV repair to accurately study the complex three dimensional cusps anatomy and their geometric interrelation with aortic root as a functional unit (Figure 3). This

is important since effective cusps height is a significant predictor of aortic valve repair failure. Indeed, an effective height > 8 mm is associated with 99.6% probability of clinically insignificant aortic regurgitation. Other significant predictors are residual regurgitation, a coaptation length < 4 mm and a level of cusp coaptation which is below the aortic annulus (type C)^[5].

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TOPIC HIGHLIGHT

- 692** Pulmonary hypertension and metabolic syndrome: Possible connection, PPAR γ and Caveolin-1
Mathew R
- 706** Transcatheter therapies for resistant hypertension: Clinical review
Lokhandwala A, Dhoble A
- 713** Exercise training in hypertension: Role of microRNAs
Neves VJ, Fernandes T, Roque FR, Soci UP, Melo SFS, Oliveira EM
- 728** Prehypertension: Underlying pathology and therapeutic options
Albarwani S, Al-Siyabi S, Tanira MO
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- 755** Genetics of coronary heart disease with reference to *ApoAI-CIII-AIV* gene region
Agrawal S, Mastana S
- 764** Hypertrophic cardiomyopathy: Can the noninvasive diagnostic testing identify high risk patients?
Zhang L, Mmagu O, Liu L, Li D, Fan Y, Baranchuk A, Kowey PR
- 771** Alcoholic cardiomyopathy
Guzzo-Merello G, Cobo-Marcos M, Gallego-Delgado M, Garcia-Garcia P
- 782** Interferon- γ and other inflammatory mediators in cardiomyocyte signaling during Chagas disease cardiomyopathy
Ferreira LRP, Frade AF, Baron MA, Navarro IC, Kalil J, Chevillard C, Cunha-Neto E
- 791** Innate immune receptors in heart failure: Side effect or potential therapeutic target?
Wagner KB, Felix SB, Riad A

- 802 Atypical presentation of acute and chronic coronary artery disease in diabetics

Khafaji HARH, Al Suwaidi JM

REVIEW

- 814 Renal sympathetic nervous system and the effects of denervation on renal arteries

Kannan A, Medina RI, Nagajothi N, Balamuthusamy S

- 824 Is reversal of endothelial dysfunction still an attractive target in modern cardiology?

Mordi I, Tzemos N

- 836 Percutaneous management of vascular access in transfemoral transcatheter aortic valve implantation

Dato I, Burzotta F, Trani C, Crea F, Ussia GP

- 847 Pseudoexfoliation syndrome and cardiovascular diseases

Andrikopoulos GK, Alexopoulos DK, Gartaganis SP

MINIREVIEWS

- 855 Management of renal artery stenosis: What does the experimental evidence tell us?

Al-Suraih M, Grande JP

- 861 Long term negative pressure ventilation: Rescue for the failing fontan?

Deshpande SR, Maher KO

- 865 Risk stratification for ST segment elevation myocardial infarction in the era of primary percutaneous coronary intervention

Brogan RA, Malkin CJ, Batin PD, Simms AD, McLenachan JM, Gale CP

CASE REPORT

- 874 Late intervention in an asymptomatic pediatric patient with anomalous left coronary artery

Lam JC, Giuffre M, Myers KA

Contents

World Journal of Cardiology
Volume 6 Number 8 August 26, 2014

APPENDIX I-V Instructions to authors

ABOUT COVER Editorial Board Member of *World Journal of Cardiology*, Ismail Biyik, MD, Associate Professor, Doctor, Department of Cardiology, Usak State Hospital, Usak 64100, Turkey

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Pulmonary hypertension and metabolic syndrome: Possible connection, PPAR γ and Caveolin-1

Rajamma Mathew

Rajamma Mathew, Section of Pediatric Cardiology, Maria Fareri Children's Hospital, Valhalla, NY 10595, United States
Rajamma Mathew, Department of Physiology, New York Medical College, Valhalla, NY 10595, United States

Author contributions: Mathew R solely contributed to this paper.
Correspondence to: Rajamma Mathew, MD, Department of Physiology, New York Medical College, Valhalla, Basic Science Building, Rm #A11, Valhalla, NY10595, United States. rajamma_mathew@nymc.edu
Telephone: +1-914-5944750

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Abstract

A number of disparate diseases can lead to pulmonary hypertension (PH), a serious disorder with a high morbidity and mortality rate. Recent studies suggest that the associated metabolic dysregulation may be an important factor adversely impacting the prognosis of PH. Furthermore, metabolic syndrome is associated with vascular diseases including PH. Inflammation plays a significant role both in PH and metabolic syndrome. Adipose tissue modulates lipid and glucose metabolism, and also produces pro- and anti-inflammatory adipokines that modulate vascular function and angiogenesis, suggesting a close functional relationship between the adipose tissue and the vasculature. Both caveolin-1, a cell membrane scaffolding protein and peroxisome proliferator-activated receptor (PPAR) γ , a ligand-activated transcription factor are abundantly expressed in the endothelial cells and adipocytes. Both caveolin-1 and PPAR γ modulate proliferative and anti-apoptotic pathways, cell migration, inflammation, vascular homeostasis, and participate in lipid transport, triacylglyceride synthesis and glucose metabolism. Caveolin-1 and PPAR γ regulate the production of adipokines and in turn are modulated by them. This review article summarizes the roles and inter-relationships of caveolin-1,

PPAR γ and adipokines in PH and metabolic syndrome.

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Key words: Adiponectin; Caveolin-1; Leptin; Metabolic Syndrome; Pulmonary hypertension; Peroxisome proliferator-activated receptor

Core tip: Pulmonary hypertension (PH) is a devastating disease with a high morbidity and mortality rate. Recent studies indicate that the metabolic alterations that occur during the course of PH have a negative effect. Importantly, PH has been observed in patients with metabolic syndrome. Caveolin-1, a membrane protein and peroxisome proliferator-activated receptor γ , a ligand activated transcription factor are abundantly expressed in vascular cells and adipocytes. They play a significant role in maintaining vascular health, and participate in glucose and lipid metabolism. Furthermore, the proximity of vasculature and adipose tissue facilitates reciprocal influence during health and disease.

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INTRODUCTION

Chronic inflammation plays a significant role in metabolic syndrome and vascular diseases including pulmonary hypertension (PH). Adipose tissue not only functions as an energy store, but also as an endocrine system producing bioactive substances that influence metabolic and vascular homeostasis. Adipocytes play an important role in regulating inflammatory response. Obesity is associated with chronic inflammation, activation of proinflammatory

cytokines, and with the infiltration of adipose tissue with macrophages and lymphocytes^[1,2]. Interestingly, increased plasma and lung levels of pro-inflammatory cytokines^[3,4] and perivascular infiltration of inflammatory cells and neo-lymphogenesis in peri-bronchial areas^[5-7] have been reported in human and experimental forms of PH. Both caveolin-1, a plasma membrane protein and peroxisome proliferator-activated receptor (PPAR) γ , a ligand-activated transcription factor belonging to the nuclear hormone receptor family are expressed abundantly in adipose and vascular tissues. They modulate inflammation, vascular contractility, cell proliferation, cell cycle progression, and play a significant role in maintaining vascular health, and participate in glucose and lipid metabolism^[8-11]. Furthermore, perivascular adipose tissue (PVAT) has been shown to modulate vascular function. Under normal circumstances, it produces relaxing factors including nitric oxide (NO), and participates in anti-contractile function^[12].

PULMONARY HYPERTENSION

A mean pulmonary artery pressure ≥ 25 mmHg constitutes PH. A number of disparate conditions are known to give rise to PH. PH is classified into 5 major clinical groups, that has recently been updated^[13]. Group 1 labeled as pulmonary arterial hypertension (PAH) includes idiopathic, heritable PAH and PAH associated with bone morphogenetic protein receptor II mutation, congenital heart defect, connective tissue diseases, portal hypertension, infection and drug toxicity. Included in this group are pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis as subcategory 1', and recently, persistent pulmonary hypertension of the newborn was assigned the subcategory 1''. The next 4 groups are labeled as PH; Group 2: PH associated with pulmonary venous hypertension secondary to left ventricular diseases, Group 3: chronic lung diseases and accompanying hypoxia leading to PH, Group 4: chronic thrombo-embolic PH and Group 5 includes miscellaneous diseases such as myeloproliferative diseases, thyroid, hematological and renal diseases. Irrespective of the underlying disease, the main features of PH are impaired vascular reactivity and remodeling, elevated pulmonary artery pressure and right ventricular hypertrophy, leading to right ventricular failure and premature death. Clinical and experimental studies suggest that the endothelial dysfunction/disruption may be an important underlying factor in the pathogenesis of PH. Importantly, endothelial dysfunction and molecular changes in pulmonary vasculature are reported to occur before the onset of PH^[14,15].

Endothelial cells (EC) are heterogeneous; they play a specialized role in the context of a specific organ. EC modulate Ca^{2+} entry, produce vascular relaxants such as NO, prostacyclin and endothelium-derived hyperpolarizing factor and maintain vascular tone, and participate in barrier function. Inflammation plays an important role in the pathogenesis of PH. EC bear the major brunt of injuries such as increased pulmonary blood flow and shear stress, inflammation, chemical/drug toxicity, ventilation-

induced injury and hypoxia resulting in endothelial dysfunction. In response to injury, EC become activated and secrete several cytokines and adhesion molecules that can affect coagulation, barrier function, and facilitate cellular adhesion and transmigration of leukocytes leading to EC dysfunction. Endothelial dysfunction leads to impaired vascular relaxation response, and the activation of proliferative and anti-apoptotic pathways, inflammatory response, and thrombogenic state leading to progressive vascular remodeling, elevated pressure and right ventricular hypertrophy^[16].

Caveolin-1 and pulmonary hypertension

In the 1950s, Palade and Yamada independently described caveolae, 50-100 nm flask shaped invaginations rich in cholesterol and sphingolipids. Caveolae are a subset of lipid rafts found on the plasmalemmal membranes of a variety of cells including endothelial, smooth muscle, epithelial cells, fibroblasts and adipocytes. Caveolae serve as a platform and compartmentalize the signaling molecules that reside in or are recruited to caveolae. Caveolae are also involved in transcytosis, endocytosis, potocytosis, and in the regulation of cell proliferation, differentiation and apoptosis *via* a number of diverse signaling pathways. Three isoforms of caveolin gene family have been identified. Caveolin-3 is muscle specific, found primarily in skeletal and cardiac myocytes. Caveolin-2 co-localizes with caveolin-1 and requires caveolin-1 for its membrane localization. Caveolin-1 (22 kD) is the major constitutive protein of caveolae^[17]. Polymerase 1 and transcript receptor factor (PTRF/cavin), a caveolar coat protein, however, is required for caveolar formation and sequestration of caveolin-1 into caveolae^[18]. Caveolin-1 is expressed in terminally differentiated cells including adipocytes, EC, epithelial cells, fibroblasts and myocytes. Caveolin-1 interacts and negatively regulates proteins such as Src family of kinases, G-proteins and G-protein-coupled receptors, eNOS, integrins and several growth factor receptors; and these interactions occur through caveolin-1-scaffolding domain (CSD, residue 82-101 in caveolin-1). For optimal activation, eNOS is targeted to caveolae, and caveolin-1 inhibits eNOS through its interaction. Heat shock protein (HSP) 90 binds to eNOS in a Ca^{2+} -calmodulin-dependent manner, reducing the inhibitory influence of caveolin-1, and increasing eNOS activity. However, caveolin-1 is essential for proper eNOS activation. Caveolin-1 regulates Ca^{2+} entry into EC, which is important for eNOS activation as well as the activation of other vasodilators, prostacyclin and endothelium-derived hyperpolarizing factor^[19]. In addition, caveolin-1 regulates not only eNOS-derived NO but also eNOS-derived superoxide. It is involved in the sequestration of uncoupled eNOS; it prevents eNOS oxidase activity, and inhibits superoxide formation^[20]. Caveolin-1 keeps smooth muscle cells (SMC) in quiescence; and it modulates Ca^{2+} regulatory molecules, increases Ca^{2+} mobilization and facilitates contractile response to agonists. Disruption of caveolin-1 has been shown to reduce myogenic tone and impair contractile responses to several agonists^[21,22]. The dynamic interrelationship

between caveolin-1 and eNOS is critical for vascular homeostasis.

In several experimental models, the loss of endothelial caveolin-1 and the reciprocal activation of proliferative and antiapoptotic pathways such as PY-STAT3, cyclin D1 and Bcl-xL have been shown to occur before the onset of PH. The rescue of caveolin-1 inhibits the proliferative pathways and attenuates PH^[15,23,24]. Besides, the mutation of caveolin-1 gene in humans is reported to be associated with PH^[25]. Studies with caveolin-1 knockout mice have further highlighted the importance of caveolin-1 in pulmonary vasculature. The re-expression of endothelial caveolin-1 has been shown to attenuate PH, vascular dysfunction and cardiomyopathy in these mice^[26]. Increased expression of PDGF-R β , the activation of PY-STAT3 and its downstream signaling pathways, cyclin D1 and Bcl-xL have been reported in pulmonary arteries from patients with PH as well as in the MCT and hypoxia models of PH^[24,27-29]. The activation of PY-STAT3 is essential for PDGF-induced cell proliferation; and the inhibition of the PDGF receptor suppresses cell proliferation *via* the inactivation of STAT3 signaling^[30,31]. Importantly, caveolin-1 acts as a suppressor of cytokine signaling, and inhibits PY-STAT3 activation and modulates proinflammatory cytokines^[32] and it inhibits other proliferative pathways including PDGF-R β , cyclin D1, Bcl-xL. It promotes cell cycle arrest *via* a p53/p21^{waf1/cip1}-dependent mechanism and regulates apoptosis by inhibiting survivin^[33,34].

In the monocrotaline (MCT) model of PH, at 2 wk post-MCT, there is a significant loss of endothelial caveolin-1 associated with the activation of proliferative and anti-apoptotic pathways, PH and right ventricular hypertrophy. As the pulmonary vascular disease progresses, by 4 wk, extensive endothelial caveolin-1 loss and EC damage occur, followed by an enhanced expression of caveolin-1 in vascular SMC. This is associated with a significantly increased expression and the activity of matrix metalloproteinase (MMP) 2 that is known to participate in cell proliferation and cell migration. Normally, MMP2 is inhibited by caveolin-1; the activation of MMP2 in the presence of enhanced expression of caveolin-1 in SMC suggests that this caveolin-1 may have lost its inhibitory function^[15]. Enhanced expression of caveolin-1 in SMC has been reported in patients with idiopathic PAH, PAH associated with congenital heart defect and drug-toxicity^[35-37]. Pulmonary arterial SMC from idiopathic PAH revealed not only enhanced expression of caveolin-1, but also Ca²⁺ dysregulation and increased DNA synthesis which could be blocked by silencing caveolin-1^[35]. This caveolin-1 in SMC becomes pro-proliferative, and facilitates cell proliferation and migration. The about face of caveolin-1 function in PH is not unlike what has been reported in cancer^[17]. The effect of caveolin-1, thus, may depend on its location, conformation, state of the disease and cell context.

PPAR γ and pulmonary hypertension

PPARs constitute a subfamily of nuclear receptors, the

master transcriptional regulators of nutrient metabolism and energy homeostasis. Three isoforms of PPAR have been identified (α , β/δ and γ). PPAR α is thought to regulate fatty acid oxidation and glucose homeostasis, and is predominantly found in liver, muscle and kidneys. Recent studies have shown that PPAR β/δ agonists relax pulmonary and mesenteric arteries independent of cGMP and cAMP mechanisms. PPAR γ is expressed in several types of tissue, including adipocytes, EC and SMC. It is an important regulator of genes involved in cell differentiation, cell growth, inflammation and angiogenesis. It forms an obligatory heterodimer with another nuclear receptor, retinoid-X-receptor which binds to peroxisome proliferator response elements that is located in the regulatory domains of genes^[38,39]. PPAR γ inhibits the production of chemokines in EC and the activation of NF κ B^[40]. In addition, it inhibits inter cellular adhesion molecules (ICAM) and vascular cellular adhesion molecules (VCAM)^[41]. Furthermore, PPAR γ increases NO production from EC and regulates superoxide generation at the EC membrane^[42,43]. PPAR γ has also been shown to reduce vascular SMC proliferation and migration^[44]. In an arterial injury model, PPAR γ was shown to have attenuated neointimal hyperplasia by modulating protein kinase G^[45]. Reduction in the expression of PPAR γ has been reported in human PAH and several experimental forms of PH such as vascular endothelial growth factor (VEGF) receptor blocker + hypoxia^[46] and a shunt model^[47]. Endogenous ligand 15-deoxy- Δ (12,14) prostaglandin J2 and thiazolidinedione (TZD) compounds used in the treatment of diabetes activate PPAR γ . Interestingly, TZD compound has been reported to attenuate the hypoxia-induced PH in mice^[48]. However, PPAR γ has also been shown to increase plasminogen activator inhibitor type-1 expression in EC which can affect vascular disease adversely^[49]. PPAR γ within the atheromatous lesion has a propensity to facilitate angiogenesis^[50]. Furthermore, PPAR γ not only upregulates caveolin-1 expression but also promotes some forms of cancer^[51,52]. PPAR γ does play an important role in vasculature but its effects may depend on the state of disease and the cellular context; and the activation of PPAR γ may not be effective in all forms of PH.

Pulmonary hypertension and associated metabolic alterations

Metabolic alterations that occur in PH negatively impact the disease. In PH, mitochondrial metabolic shift from oxidative phosphorylation to glycolytic pathway has been shown to occur in pulmonary vasculature as well as in the right ventricle. When this shift occurs in aerobic conditions, it is termed “Warburg effect” which leads to the down regulation of mitochondrial glucose oxidation. It is accompanied by fragmented, hyperpolarized mitochondrial reticulum, decreased superoxide dismutase2, metabolic shift, increased hypoxia inducible factor (HIF)-1 α , and the activation of pyruvate dehydrogenase kinase^[53]. Glycolytic pathway is associated with resistance to apoptosis; an important feature of PH. EC isolated from idiopathic PAH pulmonary arteries exhibit increased glyco-

lytic rate, decreased mitochondrial DNA levels and fewer mitochondrial numbers per cell. In addition, increased glycolytic rate has also been shown to occur in the lungs of patients with idiopathic PAH^[54]. Hyperpolarization of the mitochondrial membrane is thought to be a feature of Warburg phenotype, and apoptosis is induced by the activation of voltage-gated K⁺ channel (Kv) and depolarization of mitochondrial membrane^[55]. Mitochondrial hyperpolarization is thought to be the underlying cause of the metabolic switch observed in PH. Importantly, the loss of caveolin-1 has been shown to lead to mitochondrial dysfunction, membrane hyperpolarization, and the mitochondrial production of oxidant species. Interestingly, the glycolysis inhibition abolishes the increase in oxidant species in caveolin-1 knock-down vascular EC^[56], indicating that caveolin-1 may have a key role in the regulation of oxidative stress and metabolic switch. Recent studies have shown decreased expression of mitochondrial uncoupling protein2 and increased mitochondrial potential in pulmonary arterial SMC from patients with idiopathic PAH and from experimental models of PH. Interestingly, reactive oxygen species inhibitors decrease cell proliferation in pulmonary arterial SMC with absent mitochondrial uncoupling protein2 expression^[57]. In addition, treatment with dichloroacetate that increases the mitochondrial oxidative phosphorylation has been shown not only to prevent but also to reverse MCT-induced PH^[58]. Thus, controlling metabolic dysfunction in PH may be a valuable therapeutic measure to prevent the progression of the disease or possibly to reverse it.

ADIPOSE TISSUE AND VASCULATURE

Adipose tissue produces a number of bioactive substances including leptin, adiponectin, and inflammatory cytokines such as interleukin (IL)-6, tumor necrosis factor (TNF)- α and visfatin, and proteins such as apolipoprotein E (ApoE), plasminogen activator inhibitor 1 and apelin^[59,60]. These substances influence adipose tissue and vasculature in health and disease.

PVAT surrounds blood vessels to provide support and to maintain vascular homeostasis. Close anatomical relationship between PVAT and blood vessels allows crosstalk which is essential for both vascular and metabolic homeostasis. Anti-contraction activity of PVAT is thought to be due to the release of adipose-derived relaxing factor^[61]. In addition to the adipose-derived relaxing factor, PVAT releases other vaso-active factors including adiponectin, leptin, angiotensin (1-7) and NO. Under normal conditions these factors maintain vascular function and resistance^[12]. PVAT shares common features with brown fat tissue, which is important for thermogenesis and plays a protective role^[62].

Adiponectin was initially recognized as an insulin-sensitizing factor, now it has been found to have a role in vascular homeostasis and inflammation. Adiponectin is an anti-inflammatory adipokine; its levels are reduced in obesity. Adiponectin plays a central role in the development of metabolic syndrome and atherosclerosis; both

have a low grade inflammation. Adiponectin knockout mice show an exaggerated inflammatory response and produce increased lipopolysaccharides-induced expression of VCAM-1 and ICAM-1. Treatment with adiponectin results in a dose-dependent inhibition of TNF- α -induced monocytes adhesion to EC and the expression of VCAM-1^[63]. Interestingly, adiponectin is present in vascular EC at steady state, and it has been shown to have a significant role in vascular relaxation by activating eNOS^[64], and PGI₂ synthase^[65]. High molecular weight adiponectin stimulates eNOS phosphorylation accompanied by eNOS-HSP90-Akt complex formation and increases NO production in a dose-dependent manner; and it also inhibits caspase3 activity and promotes endothelial survival^[66,67].

Adiponectin produced in perivascular tissue is highly regulated by PPAR γ . Furthermore, PVAT regulates insulin-mediated vasorelaxation in adiponectin-dependent pathway. It increases eNOS activation as well as inhibits superoxide generation. Local expression of adiponectin gene and protein is increased in the presence of oxidative stress. Under oxidative stress and in the presence of low tetrahydrobiopterin, eNOS is uncoupled and generates superoxide. Under these circumstances adiponectin may increase superoxide generation by increasing eNOS activation^[68,69].

Removal of PVAT has been shown to enhance neointima formation; and the local but not the systemic administration of adiponectin reduces neointima formation^[70]. Obesity-induced inflammation causes increased production of pro-inflammatory adipokines and reduction in anti-inflammatory adiponectin, which contribute to pathological vascular remodeling in response to injury. Deletion of adiponectin in mice leads to PH, perivascular inflammatory infiltrates and the upregulation of E selectin^[71]. Recent studies have shown increased plasma levels of adiponectin associated with endothelial dysfunction in diabetic nephropathy^[72]. This suggests that the adiponectin levels increase in response to endothelial dysfunction and that the endothelial integrity may be necessary for normal adiponectin function.

Leptin, primarily expressed by adipocytes is involved in energy expenditure and plays a key role in inhibiting food intake and improving insulin sensitivity. In obese patients, the circulating leptin levels are high but they exhibit resistance to the effects of leptin. Congenital leptin deficiency is associated with marked obesity and hypogonadism^[73]. Increased risk of cardiovascular diseases has been reported in obese patients with elevated levels of leptin. Leptin is considered a link between metabolic disorders and immune responses. Usually, leptin increases during the course of acute infection and inflammation. Leptin has been shown to have a direct effect on T lymphocyte type 1 helper response, and leptin alters T regulatory (Treg) response. Defective leptin receptor signaling in Treg cells reduces the development of atherosclerosis^[74]. Leptin negatively affects the generation and the proliferation of the Treg cells^[75], and it promotes chronic autoimmune disorders by regulating Treg cells and their

function^[76].

Leptin receptors are expressed in EC, SMC and macrophages. Leptin induces vasoconstriction *via* the stimulation of sympathetic activity; and depending on intact and functional EC, it has a direct vasodilatory effect *via* NO release. In systemic hypertensive rats, a reduction in leptin levels is accompanied by a loss of perivascular anti-contractile function secondary to the impaired activation of eNOS^[77]. In contrast, obesity-induced increased expression of leptin enhances neointima formation. Even in the absence of obesity and increased circulating levels of leptin, overexpression of leptin in PVAT facilitates neointima formation^[78]. In cell culture studies, leptin has been shown to induce vascular SMC proliferation and migration^[79]. Furthermore, pulmonary arterial EC from patients with PAH, and PAH associated with scleroderma secrete leptin. In addition, Treg cells from these patients exhibit increased expression of leptin receptor (ObR) on the membrane^[80], indicating that leptin may have a significant role in the pathogenesis and progression of PH.

ApoE is primarily produced in liver, but other cells such as adipocytes and macrophages also produce it, but not the preadipocytes^[60]. Circulating ApoE plays an important role in the metabolism of lipoproteins. Adipocytes from ApoE knockout mice are smaller. Systemic deficiency of ApoE results in impaired clearance of triglycerides and resistance to obesity^[81]. Diet-induced or leptin-deficient obesity produces a significant reduction in ApoE expression in adipocytes. Inflammatory cytokines such as TNF- α and reactive oxygen species suppress ApoE expression, whereas systemic administration of PPAR γ increases ApoE expression. Interestingly, ApoE colocalizes with caveolin-1 in adipocytes, and the loss of ApoE results in the alterations in caveolar lipid composition and a significant reduction in caveolin-1 mRNA expression. Endogenous expression of ApoE preserves caveolar composition in adipocytes^[82,83]. ApoE is not produced in EC, but macrophage-related ApoE is internalized by EC. ApoE increases the endothelial NO production by modulating caveolin-1/eNOS interaction and it suppresses endothelial activation, and inhibits VCAM-1 expression *via* eNOS stimulation and NO production. Interestingly, ApoE has been shown to co-precipitate with caveolin-1 but not with eNOS. Deficiency of ApoE is associated with hypercholesterolemia, and the loss of its effect on eNOS activation leads to endothelial dysfunction^[84,85]. Ablation of caveolin-1 in ApoE knockout mice has shown to be protective against atherosclerosis^[86]. However, PPAR γ -induced increase in caveolin-1 expression in ApoE knockout mice confers protection against atherosclerosis^[87]. The opposing effects of caveolin-1 may be dependent on its location and conformation. Interestingly, male ApoE knockout mice on high fat diet and associated insulin resistance have been shown to develop PH, which can be reversed by PPAR γ activation^[88].

Other bioactive substances produced by adipose tissue are visfatin and apelin. Visfatin has been shown to stimulate SMC growth and angiogenesis. Apelin causes NO-dependent vascular relaxation, but it is a potent

vasoconstrictor in endothelium-denuded vessels^[59]. The foregoing observations indicate that adipose tissue, especially PVAT possesses direct vascular protective effects which are reduced or lost in obesity, resulting in an increased incidence of vascular diseases. Even in the absence of obesity, but in the presence of alterations in the balance of bioactive substances produced by PVAT can significantly influence the state of the vasculature.

METABOLIC SYNDROME

Adipose tissue has a critical role in energy balance and insulin sensitivity. A complex network of transcription factors is involved in adipogenesis. White adipose tissue is the predominant type in adults and it functions as a storage depot for energy; whereas the brown adipose tissue generates heat through mitochondrial uncoupling of lipid peroxidation. Adipose tissue consists of adipocytes, preadipocytes, leukocytes, macrophages and EC. Adipocytes are an active metabolic organ that secretes a number of adipokines including leptin, adiponectin and resistin, and are involved in glucose and lipid metabolism, energy homeostasis; and it modulates inflammation and vascular reactivity. In addition, adipose tissue secretes proinflammatory cytokines such as IL-6, IL-1, TNF- α and CC-chemokine ligand 2^[89-92].

Inflammation plays a significant role in metabolic syndrome, and the adipocytes are considered the primary site of inflammation. Metabolic syndrome includes a number of alterations such as increased waist circumference, systemic hypertension, increased levels of glucose, and impaired cholesterol and triglyceride metabolism. The major categories included in the metabolic syndrome are obesity, disorders of adipose tissue and insulin resistance. There is a positive correlation between cardiovascular diseases and the components of metabolic syndrome such as abdominal obesity, atherogenic dyslipidemia, insulin resistance with or without glucose intolerance, and the presence of pro-inflammatory and pro-thrombogenic factors^[93].

Recent studies show that EC play a key role in metabolic homeostasis. VEGF-B interacts with endothelial VEGF receptor1 also known as FLT1, and regulates endothelial transport of fatty acids into cardiac and skeletal muscle. Over expression of VEGF-B can lead to mitochondrial dysfunction, altered cardiac lipid metabolism and hypertrophy, and insulin resistance. Mice lacking VEGF-B have been shown to display decreased fatty acid uptake and lipid deposition in muscle cells. Furthermore, VEGF-B inhibition improves insulin sensitivity^[94-96]. In addition to VEGF-B, PPAR γ and apelin also have a role in fatty acid uptake by EC and coordinate it with the energy demand and to accommodate energy needs during fasting^[97].

Caveolin-1 and metabolic syndrome

Caveolin-1 in adipocytes plays an important role in glucose and lipid metabolism. Insulin receptor (IR) colocalizes with caveolin-1, and caveolin-1 stabilizes IR- β sub-

unit at the cell membrane. It stimulates IR signaling and linking insulin action to glucose uptake. Insulin recruits glucose transporter (GLUT) 4 for glucose uptake and caveolin-1 is required for its internalization after insulin removal^[98-101]. Thus, caveolin-1 plays an important role in the control of insulin signaling and facilitates GLUT4-mediated glucose uptake.

Leptin has been shown to increase the expression of caveolin-1 in adipocytes and EC, and in contrast, caveolin-1 impairs leptin signaling which in part may be responsible for inducing leptin resistance and endothelial dysfunction^[102,103]. Interestingly, patients with obesity and obesity-associated type 2 diabetes, exhibit increased expression of caveolin-1 mRNA. This increase in caveolin-1 mRNA is associated with an increased expression of inflammatory markers such as leptin, C-reactive protein, MCP-1 and TNF- α ^[104]. In diabetic mice, increased expression of caveolin-1 mRNA and protein has been shown to be associated with impaired endothelium-dependent relaxation response despite normal eNOS expression^[105]. It is likely that caveolin-1 forms a tight complex with eNOS inhibiting its activation, not unlike what is seen in the hypoxia-induced PH. In the hypoxia model of PH, the disruption of cholesterol results in the separation of caveolin-1 and eNOS resulting in increased NO production^[106].

Caveolae are also the site of fatty acid entry. The enzymes involved in *de novo* synthesis of triacylglycerol from fatty acids, and glycerol-3 phosphatase are localized in the subclass of caveolae in the plasma membrane of primary adipocytes^[107]. Caveolin-1 regulates triglycerides, lipoprotein metabolism and cholesterol homeostasis, and participates in lipid storage *via* transcytosis and also in its breakdown. In addition, it targets the lipid droplet accumulation in the cells. In atherosclerosis, caveolin-1 has been shown to promote cholesterol accumulation *via* transcytosis across EC, thus, negatively impacting the disease. Loss of caveolin-1 leads to decreased lipid accumulation resulting in progressive white adipose tissue atrophy^[108-110]. Recent studies have shown caveolin-1 gene mutations to be associated with the atypical and severe forms of lipodystrophy and hypertriglyceridemia^[111,112]. Furthermore, mutation of PTRF associated with a reduction in caveolin has been reported in patients with generalized lipodystrophy and muscular dystrophy^[113]. Loss of caveolin-1 causes significant metabolic alterations, increased glucose production in the liver and metabolic inflexibility. Metabolic flexibility is the function of adjusting the changing nutrient availability. Adiponectin has been thought to provide the metabolic flexibility. Interestingly, caveolin-1 knockout mice exhibit low circulating adiponectin despite increased mRNA and intracellular adiponectin^[114].

Studies with caveolin-1 knockout mice have revealed the importance of caveolin-1 in maintaining vascular and metabolic homeostasis. Caveolin-1 knockout mice exhibit PH and cellular hyperplasia in the lungs, cardiomyopathy, and metabolic deregulation. These mice are found to be resistant to diet-induced obesity, but have hypertriglyceridemia and develop insulin resistance on normal diet^[98]. In

addition, they exhibit increased macrophage infiltration, increased capacity for IL-6 production and an increased collagen deposition leading to increased fibrosis. Adipose tissue from these mice show increased lipolysis^[115]. Re-expression of endothelial-specific caveolin-1 ameliorates cardiopulmonary changes, but has no effect on the lack of caveolin-1 in adipocytes that accounts for lipoatrophy. The endothelial-specific caveolin-1 expression, however, limits the macrophage extravasations into adipose tissue^[116], indicating a significant role of endothelial caveolin-1 in modulating adipocytes-driven inflammatory response.

PPAR γ and metabolic syndrome

Adipose tissue especially the white adipose tissue is the major site for PPAR γ expression. PPAR γ is required for adipocytes differentiation. Activation of PPAR γ in fibroblastic cells leads to cell differentiation and lipid accumulation; and in addition, these cells acquire genes characteristic of fat cells^[117]. PPAR γ is expressed to a lesser degree in insulin target tissues such as liver and skeletal muscle. Muscle-specific PPAR γ is critical for maintaining the whole body response to insulin. The loss of muscle-specific PPAR γ leads to obesity and insulin resistance^[118]. In addition, targeted EC deletion of PPAR γ plays an important role in insulin resistance and hyperlipidemia-mediated hypertension^[119].

Impaired PPAR γ function is implicated in a number of metabolic disorders such as type2 diabetes, obesity and lipodystrophy. In humans, mutation of PPAR γ leads to obesity and severe insulin resistance. Overexpression of this mutant gene in murine fibroblasts leads to accelerated differentiation into adipocytes and increased cellular accumulation of triglycerides^[120]. PPAR γ mutation is reported to be associated with insulin resistance, diabetes and hypertension^[121], and also in cases of lipodystrophy associated with activated renin-angiotensin system and ensuing oxidative stress and hypertension^[122]. Defect in PPAR γ expression plays a significant role in PH as well as in the pathogenesis of fibrosis; importantly, scleroderma exhibits both these features^[123]. The anti-fibrotic activity of PPAR γ is thought to be mediated by hepatocyte growth factor and adiponectin. Adiponectin, an anti-inflammatory adipokine and a fat-specific target of PPAR γ prevents hepatic fibrosis in mice^[124] and hypoxia-induced PH^[125]. The administration of leptin, a proinflammatory adipokine has been found to reduce the expression and activity of PPAR γ in human lung fibroblasts and to augment TGF β -mediated fibro-proliferative response. Furthermore, the loss of leptin prevents bleomycin-induced lung fibrosis in mice^[126].

PPAR γ inhibits the production of adipokine/cytokines such as resistin, IL-6 and TNF- α , all known to promote insulin resistance. PPAR γ agonist-induced adiponectin levels are reported to be low in type 2 diabetes^[127]. Adiponectin increases fatty acid oxidation in liver and skeletal muscle, resulting in improved insulin sensitivity in skeletal muscle, and decreased glucose production in the liver, thus, leading to the reduction in circulating glucose,

free fatty acid and triglycerides^[128]. These results suggest a protective role of PPAR γ , and the crosstalk between PPAR γ and adipokines determines the progression of a given metabolic/vascular disease process. PPAR γ activators, TZD group of drugs have been used clinically to treat type 2 diabetes. TZDs increase the expression of proteins required for insulin signaling, and also reduce the circulating levels of low density lipoproteins and triglycerides. Furthermore, they attenuate the production of inflammatory mediators^[129,130]. However, TZDs are also reported to have side effects such as increased fluid retention, increased risk of congestive heart failure, decrease in bone mineral density and fractures. Selective PPAR γ modulator in experimental studies has been shown not only to increase insulin sensitivity but also to improve bone density^[131,132]. Selective PPAR γ modulation, thus, may significantly reduce the side effects of TZD.

Metabolic syndrome and pulmonary hypertension

Obesity is reported to be associated with PH, but the prevalence of PH in obesity is not known. The echocardiographic studies in 3790 normal subjects revealed higher pulmonary artery pressure to correlate with age, body mass index and gender; the incidence being higher in males^[133]. Importantly, higher frequency of obesity, diabetes and hyperlipidemia was found in patients with precapillary PH^[134]. Furthermore, obesity is a risk factor in patients with elevated pulmonary venous pressure and preserved left ventricular ejection fraction^[135].

Diabetes is reported to be associated with PH independent of coronary artery disease and congestive heart failure^[136], and insulin resistance is more prevalent in female patients^[137]. Recent REVEAL registry analysis showed a high incidence of obesity (M:F, 31%:34%) among patients with PAH; and associated comorbidities such as diabetes and chronic obstructive pulmonary disease had a negative impact on prognosis^[138,139]. In experimental studies, diabetes associated with moderate hypoxia is reported to exhibit significant endothelial dysfunction, elevated pulmonary artery pressure and RVH. It was diabetes and not the moderate hypoxia that was found to be responsible for endothelial dysfunction^[140]. These observations suggest that obesity and insulin resistance negatively impact PH.

HYPOXIA, PULMONARY HYPERTENSION AND METABOLIC SYNDROME

HIF-1 α , an O₂ sensor is a subunit of a family of HIF transcription factors. HIF-1 α regulates numerous genes involved in adaptive responses to hypoxia and modulates metabolism, growth and angiogenesis; and promotes adaptation and cell survival under hypoxic condition. VEGF, critical for angiogenesis, is one of the target genes of HIF-1 α ^[141]. Under normoxic conditions HIF-1 α is degraded. Evidence is accumulating to suggest that reactive oxygen species (ROS) generated by mitochondrial complex III is required for HIF-1 α activation and stabili-

zation; and in turn HIF-1 α activation prevents increased production of ROS in hypoxic cells^[142]. Under hypoxic conditions, cells depend on glycolysis for ATP production; and HIF-1 α is necessary for metabolic switch during hypoxia^[143]. Destabilization of HIF-1 α has a negative impact on cell and tissue adaptation to hypoxia.

HIF-1 α has been implicated in the pathogenesis of PH. HIF-1 α plays a role in cell proliferation, angiogenesis, and participates in vascular remodeling. In plexiform lesions, the proliferating EC have been shown to express HIF-1 α , its target gene VEGF and VEGF receptor 2^[144]. Recent studies have shown that the deletion of HIF-1 α in SMC attenuates hypoxia-induced PH and vascular remodeling^[145]. In some types of cancer cells, HIF-1 α under hypoxia conditions upregulates caveolin-1 and promotes ligand-independent activation of epidermal growth factor receptor, and increases cell proliferation and cell migration^[146]. Interestingly, HIF-1 α has also been shown to maintain pulmonary vascular tone during hypoxia and normoxia by decreasing myosin light chain phosphorylation; and the lack of HIF-1 α increases pulmonary vascular tone^[147]. In addition, the loss of HIF-1 α in SMC from systemic vessels causes systemic hypertension and an exaggerated response to angiotensin II. HIF-1 α is reported to decrease the expression of angiotensin II receptor type 1. Importantly, the HIF-1 α -induced decrease in the expression of angiotensin II receptor type 1 is mediated by PPAR γ ^[148]. In addition, HIF-1 α has been shown to play a protective role in the adaptation of the heart and aorta to pressure overload by regulating TGF- β signaling in EC^[149].

HIF-1 α is an important regulator of glucose transport by altering GLUT1 expression in EC. Absence of HIF-1 α is associated with significant defect in glucose uptake. Reduced glucose uptake in HIF-1 α -deficient EC can be rescued by increased expression of GLUT1 DNA, underscoring the critical role played by HIF-1 α in glucose metabolism^[150], and that the vascular dysfunction may contribute to abnormal glucose handling. Hyperglycemia has been shown to impair hypoxia-dependent stabilization of HIF-1 α ^[151]. Both hyperglycemia and hypoxia are known to occur in diabetes. Hyperglycemia-induced destabilization of HIF-1 α negatively affects the tissue adaptation to hypoxia, resulting in complications such as diabetic retinopathy, cardiovascular and renal diseases^[152]. In addition, deficiency of HIF-1 α has been shown to block stromal derived factor1 and impair mobilization of bone marrow-derived angiogenic cells, thus adversely affecting wound healing^[153]. Interestingly, hypoxia has been shown to cause insulin resistance and the inhibition of HIF-1 α in adipose tissue improves insulin resistance^[154]. Thus, both in PH and metabolic syndrome, the role of HIF-1 α may depend on the cells, disease state and the interaction of HIF-1 α with other factors including caveolin-1 and PPAR γ .

CONCLUSION

Caveolin-1 and PPAR γ are abundantly expressed in EC and adipocytes. Under normal conditions, caveolin-1 and

PPAR γ interact with adipokines (pro- and anti-inflammatory) and form a complex network to maintain metabolic and vascular homeostasis. Genetic mutations of caveolin-1 and PPAR γ lead to vascular and metabolic diseases. PVAT has a direct role in maintaining vascular reactivity. Disruption of PVAT results in the loss of anti-inflammatory and anti-contractile factors leading to endothelial dysfunction. The initial loss of endothelial caveolin-1 results in the activation of proliferative pathways leading to vascular remodeling and PH. As the disease progresses, SMC develop enhanced expression of caveolin-1. This caveolin-1 becomes pro-proliferative and participates in cell proliferation and cell migration. In adipose tissue, the loss of caveolin-1 is associated with dysregulation of insulin and lipid metabolism. However, increased levels of caveolin-1 in diabetes and hypercholesterolemia result in eNOS dysfunction. Loss of PPAR γ leads to vascular and metabolic diseases. Interestingly, PPAR γ within the atheromatous lesion facilitates angiogenesis. Adiponectin, regulated by PPAR γ increases insulin sensitivity, inhibits inflammation and facilitates NO production, thus, plays an important role in maintaining vascular and metabolic homeostasis. Leptin, a proinflammatory adipokine has an important role in food intake and energy conservation. Under normal conditions, leptin has a vasodilatory effect. However, obesity-induced increased levels of leptin cause endothelial dysfunction. It increases caveolin-1 expression which in turns inhibits leptin.

Vasculature and adipose tissue owing to their proximity share the complex network of transcription factors, and influence each other in health and disease. The network of these factors is rather complex and delicate, which can be deregulated by injury and/or inflammatory process leading to a stage where the cytoprotective factors become cytotoxic depending on the state of the cell/organ. Rudolf Virchow (1821-1902) a German physician is reported to have said “The body is a Cell State in which every cell is a citizen. Disease is merely the conflict of citizens of the State brought about by the action of an external force”. It is not difficult to imagine that this conflict can easily spill into the neighboring organs/systems.

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WJC 6th Anniversary Special Issues (1): Hypertension

Transcatheter therapies for resistant hypertension: Clinical review

Adil Lokhandwala, Abhijeet Dhoble

Adil Lokhandwala, Department of Internal Medicine, University of Arizona College of Medicine at South Campus, Tucson, AZ 85713, United States

Abhijeet Dhoble, Department of Cardiology, Cedars Sinai Medical Center, Los Angeles, CA 90048, United States

Author contributions: Lokhandwala A designed and wrote the manuscript, reviewed the manuscript and corrected the manuscript for its final presentation; Dhoble A reviewed the manuscript and corrected the manuscript for its final presentation.

Correspondence to: Abhijeet Dhoble, MBBS, MPH, FACP, Department of Cardiology, Cedars Sinai Medical Center, 8700 Beverly Blvd, Room 2S03G-2, Los Angeles, CA 90048, United States. abhijeetdhoble@gmail.com

Telephone: +1-310-2486719 Fax: +1-310-4230127

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Abstract

Resistant hypertension (RHTN) is a commonly encountered clinical problem and its management remains a challenging task for healthcare providers. The prevalence of true RHTN has been difficult to assess due to pseudoresistance and secondary hypertension. Atherosclerotic renal artery stenosis (RAS) has been associated as a secondary cause of RHTN. Initial studies had shown that angioplasty and stenting for RAS were a promising therapeutic option when added to optimal medical management. However, recent randomized controlled trials in larger populations have failed to show any such benefit. Sympathetic autonomic nervous system dysfunction is commonly noted in individuals with resistant hypertension. Surgical sympathectomy was the treatment of choice for malignant hypertension and it significantly improved mortality. However, post-surgical complications and the advent of antihypertensive drugs made this approach less desirable and it was eventually abandoned. Increasing prevalence of RHTN in recent decades has led to the emergence of minimally invasive interventions such as transcatheter renal

denervation for better control of blood pressure. It is a minimally invasive procedure which uses radiofrequency energy for selective ablation of renal sympathetic nerves located in the adventitia of the renal artery. It is a quick procedure and has a short recovery time. Early studies in small population showed significant reduction in blood pressure. The most recent Symplicity HTN-3 study, which is the largest randomized control trial and the only one to use a sham procedure in controls, failed to show significant BP reduction at 6 mo.

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Key words: Resistant hypertension; Renal denervation; Renal artery stenosis; Renal artery stenting; Transcatheter therapy; Sympathetic autonomic nervous system

Core tip: The aim of this paper is to review resistant hypertension (RHTN), including primary and secondary causes. Renal artery stenosis is one of the secondary cause of RHTN but angioplasty and stenting of renal artery for management of RHTN has failed to show any benefit. Sympathetic nervous system dysfunction is commonly noted in individuals with resistant hypertension. Renal sympathetic nerve denervation is a minimally invasive procedure which may help improve management of RHTN. However, the Symplicity HTN-3 trial failed to show a meaningful reduction in BP and has questioned this approach.

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INTRODUCTION

Resistant hypertension is defined as above goal systolic

blood pressure (SBP) despite therapy with three or more antihypertensive medications of different classes at maximum tolerable doses with one being a diuretic^[1]. The definition can be extended to at goal blood pressure (BP) requiring four or more drugs of different classes^[1]. The true prevalence of resistant hypertension (RHTN) is difficult to assess due to significant number of patients with poor medical compliance and/or suboptimal treatment regimen^[1]. Prevalence of RHTN according National Health and Nutritional Examination Survey (NHANES) is 8.9% within the hypertension population^[2]. With rising incidence of obesity, and people living longer, it is likely to become a major public health concern in the upcoming decades^[1]. RHTN should be considered after excluding pseudo-hypertension and secondary causes of hypertension. It is associated with significant end organ complications including, coronary artery disease (CAD), stroke and chronic kidney disease (CKD). Prognosis is poor in individuals who have failed therapy with multiple classes of antihypertensives. The degree of reversibility of end organ damage with successful control of BP in these individuals is lacking evidence, but optimal blood pressure control in general has shown to delay onset and progression of end organ complications and it reduces the incidence of major vascular events^[1]. RHTN is beginning to become a global issue, which has led to the advent of minimally invasive interventions for optimal BP control.

INITIAL DIAGNOSIS OF RHTN

RHTN is a diagnosis of exclusion. The initial step in management of poorly controlled blood pressure would be to rule out pseudo-resistance and secondary causes of HTN. Poor BP measurement technique, and use of improper cuff size can lead to falsely elevated BP readings. This can be avoided by allowing a patient to sit in a quiet room for a few minutes before checking BP, using an appropriately sized cuff and proper technique^[1]. Medical noncompliance is another commonly encountered problem and has been noted in up to 40% of newly diagnosed hypertensive patients^[1]. White coat hypertension is present in 20% to 30% of individuals and it should be further evaluated with ambulatory BP measurement^[1]. Lifestyle factors such as obesity, excessive dietary salt intake, heavy alcohol consumption and certain medications can significantly contribute to elevation of BP, and it must be addressed before giving diagnosis of RHTN^[1]. The most common secondary causes of RHTN are RAS, obstructive sleep apnea (OSA), primary hyperaldosteronism and renal parenchymal disease^[1]. Fibromuscular dysplasia is a common cause of RAS in middle aged females, whereas atherosclerotic RAS is predominantly seen in the elderly. OSA is a known cause of hypertension and its severity is directly associated with difficulty in controlling BP^[1]. OSA is thought to cause sympathetic dysregulation which can lead to RHTN^[1]. Primary hyperaldosteronism has a prevalence of 20 percent in individuals with RHTN and its etiology can be often obscure^[1]. CKD is commonly

the result of long standing poorly controlled HTN and it can lead to RHTN.

RENAL ARTERY STENOSIS AS SECONDARY CAUSE OF RHTN

RAS is often noted in individuals with RHTN. Stenting or angioplasty in addition to optimal medical management for atherosclerotic RAS has failed to show any significant benefit in regards to HTN or CKD in randomized control trials (RTC)^[3]. Up to 90% of renal artery stenosis in the elderly population is due to atherosclerosis^[1,3,4]. A significant degree of RAS can decrease renal perfusion which leads to the over-activation of the renin-angiotensin-aldosterone axis (RAAS)^[4]. RAAS over-activation leads to increase in sodium and water retention, causing elevation in systemic blood pressure^[4]. The severity of stenosis required to cause over activation of RAAS is unknown, but use of ACE-inhibitor can cause acute worsening of renal function and should raise suspicion of significant RAS in these individuals^[4]. There is also up-regulation of SANS which can further make it difficult to control BP^[4]. Such individuals are at high risk of end organ complications including left ventricular hypertrophy, heart failure with recurrent pulmonary edema and CKD^[4].

TRANSCATHETER THERAPY FOR ATHEROSCLEROTIC RENAL ARTERY STENOSIS

Theoretically, stenting of the stenotic lesion should resolve RHTN. Initial studies showed significant reduction in SBP and this led to increase in revascularization rates for renal artery stenosis^[3,4]. However, recent RCT have shown such revascularization to be futile^[3,4]. The “Blood pressure outcome of angioplasty in atherosclerotic renal artery stenosis trial”, aka. EMMA trial, concluded that previous uncontrolled and unblinded studies had over-estimated the benefits of renal artery revascularization^[5]. No significant difference in mean 24-h ambulatory blood pressure was noted between the control group and angioplasty group at the end of 6 mo^[5]. “The Randomized comparison of percutaneous angioplasty *vs* continued medical therapy for hypertensive patients with renal artery stenosis trial” was a randomized study that enrolled patients with renal artery stenosis of 50% or greater and minimum diastolic BP of 95 on at least two antihypertensive medications^[6]. Revascularization resulted in modest systolic BP improvement without any change in renal function but, there was significant post-procedural complication noted in the intervention group^[6]. “The effect of balloon angioplasty on hypertension in atherosclerotic renal artery stenosis trial”, also known as the “Dutch renal artery stenosis intervention cooperative (DRASTIC)” concluded that the benefit of angioplasty was “little” over medical management^[7].

Table 1 Renal artery stenting/angioplasty in resistant hypertension

Ref.	Size	Follow up period	Mean SBP reduction with stenting/angioplasty	Mean SBP reduction with medical therapy	P value
Cooper <i>et al</i> ^[3] (Coral trial)	947	43 mo	16.6 ± 21.2	15.6 ± 25.8	0.03
Van Jaarsveld <i>et al</i> ^[7] (DRASTIC trial)	106	12 mo	19	17	0.51
Plouin <i>et al</i> ^[5] (EMMA trial)	49	6 mo	12 ± 20	8 ± 16	0.46
Webster <i>et al</i> ^[6]	135	3-54 mo	34	8	0.018

SBP: Systolic blood pressure.

“Cardiovascular outcomes in renal atherosclerotic lesions trial (aka, CORAL)” was an NIH funded, open-label, unblinded and a multicenter randomized study^[3]. It compared stenting *vs* medical therapy in atherosclerotic renal artery stenosis^[3]. This trial randomized 947 individuals with elevated SBP and/or CKD with estimated GFR of 60 mL/min per 1.73 m² of BSA as per MDRD formula and RAS of at least 60%^[3]. Patients were randomized to either only medical therapy or medical therapy plus renal artery stenting group^[3]. The primary endpoint of this study was a major cardiovascular or renal event^[3]. Over a 43-mo median follow up, there was no significant difference in regards to the primary endpoint between the 2 study groups^[3]. SBP was noted to be reduced in the stenting plus medical therapy group by 16.6 ± 21.2 mmHg and in the medical therapy only group by 15.6 ± 25.8^[3]. There was only a modest SBP lowering in stenting group compared to medical therapy group (-2.3 mmHg) with a Confidence Interval (CI) of -4.4 to -0.2 and *P* value of 0.03^[3]. Once again revascularization for renal artery stenosis was proven to be futile, putting another nail into its’ coffin (Table 1).

Earlier trials had a smaller sample size and the patients had clinically insignificant RAS, which question their validity. The sample size was 49 in the EMMA trial and the DRASTIC trial had 106 participants, which is too small to detect a significant difference between the study groups^[5,7]. This increases the chance of a type 2 statistical error. They enrolled patients with mild RAS when a lesion of at least around 70% is deemed to be hemodynamically significant by many experts^[8,9]. A crossover rate to therapy group was 44% in DRASTIC study which further obscures outcomes^[7]. Earlier trials assessing effect of revascularization on RHTN used angioplasty of stenotic lesions that may not be as effective as stenting^[8-10]. CORAL was one of the largest randomized trial with 947 participants comparing medical therapy *vs* endovascular stenting in addition to medical therapy for RHTN. It also included stricter criteria in regards to the degree of stenosis required to be eligible for participation, which was not seen in earlier studies. CORAL trial seems of have addressed some of the common issues with previous studies and provides the most statistically significant data.

SYMPATHETIC THEORY OF RHTN

Sympathetic autonomic nervous system (SANS) dysfunction is seen in 50% of hypertensive individuals, which makes it a promising therapeutic target^[11]. The sympathetic fibers densely innervate the kidneys and are mainly located in the adventitial layer of the vascular wall of the renal arteries^[12]. Activation of the afferent limb of the Renal SANS stimulates the posterior hypothalamus, the autonomic centers in the medulla oblongata and the mid brain^[13,14]. All messages are integrated into the autonomic centers and are relayed back to the kidneys *via* the thoraco-lumbar paravertebral ganglia, the superior mesenteric ganglia and celiac prevertebral ganglia^[13,14]. Increase in efferent sympathetic tone leads to vasoconstriction of the renal vasculature by activation of the alpha-1a receptors which leads to a decrease in blood flow to the kidneys^[15]. It accelerates alpha-1b adrenergic receptor mediated tubular reabsorption of sodium and water^[15]. It also causes over activation of the RAAS through the beta-1 adrenergic receptors located on the juxtaglomerular cells^[15]. Sympathetic over-activity on the heart increases cardiac output and its effect on blood vessels increases peripheral vascular resistance in an effort to increase renal perfusion^[13]. These pathophysiologic changes make an individual susceptible to RHTN which can lead to end organ complications over time^[13,16].

THE SURGICAL APPROACH TO SYMPATHETIC DENERVATION FOR RHTN

Surgical sympathectomy was the treatment of choice for malignant hypertension before antihypertensive medications were available^[11,16]. Five-year mortality from malignant hypertension was estimated to be 100%^[17,18]. Thoracolumbar splanchnicectomy was first introduced in 1938^[18]. Treatments ranging from radical subdiaphragmatic splanchnicectomy to less aggressive interventions such as sympathetic gangliectomy resulted in reduced blood pressure and favorable end organ changes^[11,19]. However, they were associated with undesirable adverse effects such as, orthostatic hypotension, sexual dysfunction, incontinence, anhydrosis and tachycardia^[11,19]. The surgery was typically performed as a one or two step procedure and required extended hospital stay^[17]. Surgical sympathectomy became a second line treatment after introduction of antihypertensives for patients whose BP was uncontrolled despite medical management^[17]. Surgical sympathectomy increased sensitivity of antihypertensive drugs and had lower mortality compared to medical management alone^[17]. As newer and more potent antihypertensive medications of different classes became available, this radical approach phased out due to its undesirable adverse effects. However, suboptimal control of blood pressure on maximal medical therapy, the increasing prevalence of RHTN and evidence of renal sympathetic nerve over-activity in hypertensive

individuals has sparked interest in catheter based renal sympathetic denervation as a promising therapeutic option^[16,20,21].

TRANSCATHETER RENAL DENERVATION

Transcatheter renal sympathetic nerve ablation is a minimally invasive procedure. It complements the BP lowering effects of the former radical approach without its adverse effects and has a much faster post-procedural recovery time^[13]. The post-operative mortality in patients treated with the surgical approach was as high as 11% compared to relatively none with RDN^[18]. Contraindications to RDN mainly include GFR < 45 mL/min per 1.732 m², past interventions such as angioplasty or stenting, abnormal anatomy, Diabetes type 1, age less than 18 years and pregnancy^[22]. One of the devices widely studied in RCT is the Symplicity Renal Denervation System by Medtronic. This device consists of a low power radio frequency generator and a disposable catheter^[13]. The procedure is performed under conscious sedation through percutaneous access. The catheter tip is an opaque platinum electrode. It is hand guided into the renal artery, adjacent to the dense neural site located near the renal hilum^[13]. The design of the catheter allows safe delivery of low level radio frequency energy across the arterial wall to ablate the nerves located in the adventitia of the renal artery^[13]. Multiple ablations are delivered in a circumferential pattern every few millimeters within both renal arteries to ensure complete ablation. The procedure takes less than an hour and the patient is usually observed for a day after the procedure^[13].

Many other catheter designs are currently being investigated. The ST. Jude's Enlig HTN Renal Denervation System uses a multi-electrode catheter which delivers the ablation in a specific circumferential pattern, eliminating the need for catheter manipulation and administering multiple ablations^[22]. The EnligHTN 1 trial was a non-randomized study which evaluate the efficacy and safety of this device in 46 patients whose mean office BP was 176/96 mmHg^[22]. Office BP reduced by 26/10 at 6 mo with a *P* value of < 0.0001 without any complications^[22]. The Vessix V2 renal denervation system uses an over the wire balloon catheter with electrodes in a specific pattern to deliver RF energy and it is currently being evaluated in the REDUCE-HTN trial expected to complete in December 2014^[23].

Catheter based ultrasound renal denervation is a newer technique which uses intravascular ultrasound for selective denervation of the renal nerves in the adventitia of the artery^[24]. The device uses a catheter-based transducer, which delivers high frequency sound waves in a circumferential manner^[24]. The transducer has an inflatable balloon with a water circuit that keeps the walls of the arterial lumen cool when energy is being delivered^[24]. This prevents thermal damage to the vessel wall while selectively ablating the renal nerves^[24]. The circumferential delivery of energy is not dependent on the position of the catheter which allows for a consistent post procedural

outcome^[24]. This device is currently being evaluated in 50 patients in the ACHIEVE study, which is anticipated to be complete in February 2015^[25]. Chemical renal nerve ablation is the latest technique which uses peri-adventitial dehydrated ethanol injection administered in a circumferential pattern^[26]. Most of the newer devices are "energy based" and can lead to thermal injury of the vessel wall which is an advantage of chemical RDN^[26]. This approach has been successful in lowering renal parenchymal norepinephrine levels at 2 wk in swine models which is a measure of reduced sympathetic activity^[26]. Randomized control trial in human model is needed to evaluate its safety and efficacy.

The first reported RDN in humans was done by Schlaich and Colleagues in 2009^[27]. The subject was a 59-year-old male patient with history of two TIA, untreated OSA secondary to intolerance to CPAP, and RHTN who was on seven antihypertensive medications^[27]. He underwent this procedure without any complications^[27]. Reductions were noted in renal norepinephrine spillover and mean office blood pressure, while the renal blood flow increased^[27]. "The Catheter-based renal sympathetic denervation for resistant hypertension" was a multicenter safety and proof-of-principle cohort study, which evaluated the BP lowering effect and safety of renal denervation in 50 patients from Europe and Australia^[28]. Eligible patients had an office SBP \geq 160, and were on three or more antihypertensive agents of which one was a diuretic with no previous ablations, stenosis, and bilateral kidneys with an anatomy that was conducive to the procedure^[28]. Out of the 50 patients, 45 underwent the procedure and 5 were disqualified primarily due to dual renal artery anatomy^[28]. Patients who underwent the procedure had a mean office blood pressure reduction of 27/17 at 12 mo with one complication of renal artery dissection during the procedure^[28].

The Symplicity HTN-1 trial was a major open label study with a total of 153 patients enrolled at centers in the United States, Europe and Australia^[29]. They were followed for 24 mo and were noted to have a mean BP reduction of 32/14^[29]. Statistically, *P* value for the reduction was noted to be < 0.0001 for SBP and diastolic BP (DBP) at intervals of 1, 3, 6, 12 and 18 mo, except for *P* value of = 0.002 for DBP at 24 mo^[29]. The complication rate was three percent with three patients experiencing groin access site pseudoaneurysm and one patient experiencing renal artery dissection^[29]. A final 3 year report evaluated follow up data of only 88 of the 153 patients and noted a mean SBP reduction of 32 mmHg with a 95%CI of -35.7 to -28.2^[30]. Complications over the three year period were one new renal artery stenosis which needed stenting and three unrelated deaths^[30].

The Symplicity HTN-2 was the first multicenter, prospective RCT that evaluated the effectiveness of transcatheter renal denervation. Primary end point was change in seated SBP at the six month point^[12]. A total of 106 eligible participants aged 18 to 85 years who had SBP \geq 160 mmHg or \geq 150 mmHg if patient was a type 2 diabetic despite compliance with treatment on \geq

Table 2 Renal nerve denervation in resistant hypertension

Ref.	Sample size	Follow up duration	Mean SBP reduction in RDN group (in mmHg)	Mean SBP reduction in control group (in mmHg)	P value
Worthley <i>et al</i> ^[22] (EnligHTN 1 trial)	46	6 mo	26	No randomized control group	0.0001
^a Krum <i>et al</i> ^[28]	45	12 mo	27	No randomized control group	0.001
Symlicity HTN-1 investigators ^b	153	24 mo	32	No randomized control group	0.0001
Esler <i>et al</i> ^[12] (Symlicity HTN-2)	106	6 mo	32 ± 23	+ 1	0.0001
Bhatt <i>et al</i> ^[32] (Symlicity HTN-3)	535	6 mo	14.13 ± 23.93	11.74 ± 25.94	< 0.001

^aFollow up data available for $n = 9$ at 12 mo; ^bFollow up data available for $n = 18$ at 24 mo. SBP: Systolic blood pressure; RDN: Renal denervation; HTN: Hypertension.

3 antihypertensive medications were screened^[12]. A total of 52 patients were randomized to renal denervation group at 24 participating centers in Australia, Europe and New Zealand^[12]. BP in the intervention group was reduced by 32/12 mmHg (SD ± 23/11 mmHg) from a baseline of 178/97 mmHg (P value < 0.0001) compared to change of -1/0 mmHg from baseline of 178/97 mmHg in control group (P value = 0.77 for SBP and 0.83 for DBP) with no significant post procedural complications^[12]. Thirty six month data was recently presented which showed a reduction in BP by an average of 33/14 (P value < 0.01) in 40 of the study participants^[31].

The Symlicity HTN-3 is the largest sham controlled, single blinded trial to recruit 535 patients. Inclusion criteria were SBP ≥ 160 mmHg on stable antihypertensive regimen with ≥ 3 medications of different classes at full tolerated doses with one being a diuretic^[32]. The primary endpoint was change in office SBP measurement at 6 mo and a secondary endpoint assessed 24 h ambulatory BP^[32]. Patients were randomized in a 2:1 fashion between RDN group and control group^[32]. Within the RDN group, SBP was reduced by 14.13 mmHg with a mean SD of ± 23.93 and in the control group, SBP was reduced by 11.74 mmHg with a mean SD of ± 25.94 at 6 mo (P value < 0.001 for change for baseline for both groups)^[32]. With ambulatory BP monitoring, RDN group showed a reduction in SBP by 6.75 mmHg with mean SD of 15.11 and in the control group, SBP was reduced by 4.79 mmHg with a mean SD of 17.25^[32]. The trial did meet its safety end point^[33]. Compared to former studies, Symlicity HTN-3 is the largest and the only blinded RTC which included a sham procedure in the control group. It is the first trial to show that there was no significant difference between the RDN when compared to medical management alone. Symlicity HTN-4 was also a RCT which was estimated to enroll 580 patients but was suspended after release of data from the Symlicity HTN-3 trial^[34]. It was similar to Symlicity HTN-3, but its eligibility criteria required participant to be on ≥ 3 antihypertensive medications of different classes with one of them being a thiazide or a thiazide like diuretic and SBP ≥ 140 mmHg but less than 160 mmHg^[35] (Table 2).

DISCUSSION

The long term benefits of optimum BP control on end organ prognosis is beyond doubt. Newer antihypertensive agents are increasingly selective and efficacious but the prevalence of RHTN is still a public health burden. This prevalence is likely to increase with increasing incidence of obesity and longevity. RHTN is essentially a diagnosis of exclusion and should be considered in individuals after pseudoresistance and secondary causes of HTN are ruled out. Angioplasty and stenting can successfully treat RHTN in individuals with renal artery stenosis due to fibromuscular dysplasia but it has proven to be futile in atherosclerotic RAS. Renal denervation for RHTN may be an excellent therapy with low complication rates. Rare complications such as RAS requiring stenting, renal artery dissection and access site pseudoaneurysm have been noted^[28,30]. The current safety profile of RDN is limited to 3 years and it appears to be fairly acceptable^[36]. However, long term safety of such intervention is currently unknown^[28,30]. Earlier trials presented promising results but the data from Symlicity HTN-3 trial may have brought RDN to a screeching halt for the time being. In comparison to former trials, Symlicity HTN-3 is the largest RTC, and it is the only one to include a sham group which underwent an angiography instead of denervation. Most trials used office BP reduction as primary endpoint that can vary significantly and is not as accurate as ambulatory BP monitoring. This was also addressed in Symlicity HTN-3 trial and it didn't show a meaningful SBP reduction between the two groups, thus, providing us with the most objective data on RDN. Nerve regrowth has been documented in individuals after renal transplant, questioning the durability of RDN, which is currently unknown^[28]. RDN also does not completely eliminate the need for medical management and most patient still need to continue on an oral antihypertensive medications. In the meanwhile, RDN continues to be an option after failure with lifestyle and medical management in approved markets^[36]. Is there a sub group of individuals with RHTN that may benefit from RDN? Future studies are need to address this question. Much has to be established about the efficacy and long term safety of RDN.

Any conclusions based on currently available data may be premature.

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WJC 6th Anniversary Special Issues (1): Hypertension

Exercise training in hypertension: Role of microRNAs

Vander José das Neves, Tiago Fernandes, Fernanda Roberta Roque, Ursula Paula Renó Soci, Stéphanio Freitas Soares Melo, Edilamar Menezes de Oliveira

Vander José das Neves, Tiago Fernandes, Fernanda Roberta Roque, Ursula Paula Renó Soci, Stéphanio Freitas Soares Melo, Edilamar Menezes de Oliveira, Laboratory of Biochemistry and Molecular Biology of the Exercise, School of Physical Education and Sport, University of Sao Paulo, Sao Paulo, SP 05508-900, Brazil

Author contributions: Neves VJ, Fernandes T, Roque FR, Soci UP, Melo SFS wrote part of manuscript; Neves VJ was organizer; Oliveira EM, adviser, edited this manuscript.

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Correspondence to: Edilamar Menezes de Oliveira, PhD, Laboratory of Biochemistry and Molecular Biology of the Exercise, School of Physical Education and Sport, University of São Paulo, Av. Professor Mello Moraes, 65, Cidade Universitária, São Paulo, SP 05508-900, Brazil. edilamar@usp.br

Telephone: +55-11-30912118 Fax: +55-11-38135921

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Abstract

Hypertension is a complex disease that constitutes an important public health problem and demands many studies in order to understand the molecular mechanisms involving its pathophysiology. Therefore, an increasing number of studies have been conducted and new therapies are continually being discovered. In this context, exercise training has emerged as an important non-pharmacological therapy to treat hypertensive patients, minimizing the side effects of pharmacological therapies and frequently contributing to allow pharmacotherapy to be suspended. Several mechanisms have been associated with the pathogenesis of hypertension, such as hyperactivity of the sympathetic nervous system and renin-angiotensin aldosterone system,

impaired endothelial nitric oxide production, increased oxygen-reactive species, vascular thickening and stiffening, cardiac hypertrophy, impaired angiogenesis, and sometimes genetic predisposition. With the advent of microRNAs (miRNAs), new insights have been added to the perspectives for the treatment of this disease, and exercise training has been shown to be able to modulate the miRNAs associated with it. Elucidation of the relationship between exercise training and miRNAs in the pathogenesis of hypertension is fundamental in order to understand how exercise modulates the cardiovascular system at genetic level. This can be promising even for the development of new drugs. This article is a review of how exercise training acts on hypertension by means of specific miRNAs in the heart, vascular system, and skeletal muscle.

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Key words: Exercise training; Hypertension; MicroRNA; Heart; Vascular system; Macrocirculation; Microcirculation; Muscles; Angiogenesis

Core tip: Numerous studies have shown that exercise training exerts beneficial effects on hypertension. Thus, several important studies have established links between exercise training, hypertension and the post-transcriptional regulators known as miRNAs. It is interesting to note that exercise training helps to control hypertension through these regulators, by promoting changes in the cardiovascular system towards normality. This review summarizes the way in which exercise training acts on the cardiovascular system to control the side effects of hypertension on the heart, macro- and microcirculation, and skeletal muscles.

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INTRODUCTION

Exercise training (ET) is a well-known form of preventing or reducing cardiovascular disturbances. It is able to prevent or reduces the vascular changes that are the precursors of high blood pressure, such as diminished nitric oxide (NO) availability and increased oxidative stress. It is also able to reduce sympathetic nervous system (SNS) activity and cardiac output, improve angiogenesis and reduce peripheral vascular resistance. Therefore, ET has been used as a most successful non-pharmacological therapy for the treatment of hypertensive patients. It promotes a reduction in blood pressure and helps to reduce the medication used by these patients (in some cases, it promotes discontinuation of the medication used); thereby decreasing the side effects of pharmacotherapy and the financial cost of hypertension to public health^[1]. Despite the continuous advances in options of pharmacological therapies for hypertension, it remains an important and growing public health problem worldwide, affecting more than one billion people across the planet^[2]. Today, it is estimated that it kills nine million people per year^[3]. It is in this context that ET has a high relevance in hypertension, contributing as an additional tool for the treatment or prevention of this disease.

Hypertension is a persistent elevation of systemic blood pressure with multifactorial causes. Its development is determined by a cluster of environmental factors associated with genetic susceptibility. The mechanisms by which hypertension is generated (such as hyperactivity of SNS, overactivation of the renin-angiotensin-aldosterone system, endothelial dysfunction, and others) are responsible for the gradual development of pathological manifestations in the form of vascular, cardiac and renal diseases, such as atherosclerosis, stroke, pathological cardiac hypertrophy, myocardial infarction, heart and kidney failure^[2]. Whereas, ET is able to minimize the effects of multiple factors that induce the development of hypertension, and by extension, it also helps to prevent or reduce the development of the aforementioned pathological manifestations.

ET promotes numerous cardiovascular and muscular adjustments that are antihypertensive. These adjustments depend on the amount of ET, which is determined by the volume (training time), intensity (degree of training load) and frequency of ET (number of training sessions at any given time)^[4]. In this context, aerobic exercise promotes physiological cardiac hypertrophy^[5], reduction in systolic blood pressure (SBP) and heart rate (both at rest and under submaximal loads)^[6,7], increases the lumen diameter of the coronary arteries^[8] and cardiac blood flow^[9], increases the circulating NO^[10], corrects the peripheral capillary rarefaction in hypertensive animals^[7], promotes revascularization^[11] and reduces peripheral vascular resistance. ET also promotes important meta-

bolic adaptations that reflect on blood pressure control, for example, reduction of plasma triglycerides and low-density lipoproteins, as well as increased insulin sensitivity in tissues^[12]. In addition to aerobic exercise, physical resistance training with anaerobic characteristics is also able to induce physiological cardiac hypertrophy^[13,14]. Moreover, positive effects have been shown on reducing systolic, mean, and diastolic blood pressure, and heart rate in trained when compared with untrained rats^[14].

It is interesting to note that aerobic or resistance training may promote different adaptations in the cardiovascular system, but all adaptations are beneficial to regulating the blood pressure. However it is not only the type (aerobic or anaerobic) of exercise that is important, but also the modality of exercise performed (for example running, walking, cycling and swimming)^[15]. In this case, Nualnim *et al*^[16] has shown that swimming training was able to promote hypotensive effects and improve the vascular function in adults over 50 years of age. Cycling exercise (30 min, 5 d per week, for 3 mo) significantly decreased the resting blood pressure and increased the NO plasma concentration in older (59-69 years) normotensive women, suggesting that aerobic ET exerts the beneficial effect of increasing NO production in previously sedentary older humans^[10]. Furthermore, moderate intensity walking decreased the baseline SBP of postmenopausal women with hypertension^[17], and treadmill exercise improved the endothelial function and vascular stiffness in coronary and mesenteric arteries of spontaneously hypertensive rats, which may be related to decreased oxidative stress and increased endothelial-dependent NO production^[15].

In view of the beneficial effects of ET on the treatment of hypertension, and the new genetic findings revealed in the last decades, several scientists have turned their attention to a new class of gene expression regulators, known as microRNAs (miRNAs), which have been shown to be important factors in the gene regulation of hypertension and possible therapeutic targets for this disease^[2]. The miRNAs are small, noncoding RNAs with approximately 17-25 nucleotides in length, which act as potent posttranscriptional regulators of gene expression. They can couple with sites in 3'-untranslated (3'-UTR) in the messenger RNAs (mRNAs) of protein-coding genes and negatively regulate their expression^[18-20]. The posttranscriptional regulation realized by the miRNAs in 3'-UTR is dependent on the degree of complementarity between them and the target mRNA. Thus, the miRNA does not require perfect complementarity for target recognition. Due to the fact that they have small sequences and act without the need for complete pairing^[21], a single miRNA can regulate up to 200 mRNAs, and more than one miRNA can regulate a single mRNA^[22].

As hypertension is developed on the basis of genetic susceptibility associated with environmental factors, many studies have shown associations between it and miRNAs; and others between it, miRNAs and ET as a way to prevent or minimize the harmful effects of environmental and/or genetic factors that promote hyperten-

sion. Based on the abovementioned data, the aim of this review is provide an overview of how ET can help to regulate blood pressure by means of specific miRNAs in the heart, vascular system, and skeletal muscle.

EFFECTS ON THE HEART

Hypertension is the major risk factor for congestive heart failure and chronically induces a chronic pressure overload on the heart. Sustained high blood pressure induces pathological cardiac hypertrophy (CH) and contractile dysfunction as compensatory mechanisms to reduce left ventricle wall stress. In addition to the increased size of cardiomyocytes, the growth of extracellular matrix is exacerbated and consequently there is interstitial fibrosis, and abnormalities occur in the systemic and coronary vasculature^[23].

Whereas, ET consists of a frequent, but intermittent stimulus of hemodynamic volume overload on the heart, which induces physiological CH. In this condition, the increase in size of the cardiomyocytes predominantly occurs by expression of sarcomere proteins, and the process is concatenated with preserved or improved cardiac function^[20]. Indeed, it is known that ET is able to decrease systolic and diastolic blood pressure in hypertensive humans and rats, and that the physiological CH or pathological CH triggers different signaling pathways, which in turn trigger specific transcription factors. Consequently, the pattern of gene expression is different in the two types of CH^[24-26]. In addition, there is an intricate network of transcriptional and posttranscriptional mechanisms involved in the differential expression of these genes, and there is still much to clarify as regards the differentiation of physiological and pathological phenotypes of CH^[26].

The miRNAs are part of the posttranscriptional mechanism, performing negative regulation of several target mRNAs involved in both physiological and pathological CH. The miRNAs are essential in different cell processes involved in the regulation of cardiovascular phenotypes, such as cardiomyocyte growth, remodeling, interstitial fibrosis, and heart failure. Several studies that postulate the relations between CH, hypertension and miRNAs have emerged. The miRNAs more frequently cited in cardiomyocytes studies are the miRNA-1, -133, -30, -21, -98, -378, -221, -22, -27, -212/132, -199 and -350 with several targets that are involved in the adaptive response of CH^[27,28].

Recent studies have supported the suggestion that CH may be caused by inflammatory signaling, and that this may be mediated by miRNAs. The miRNA-155 is expressed in macrophages and is a key mediator of cardiac injury in hypertensive heart disease, by the regulation of cardiac inflammation, dysfunction and hypertrophy in pressure overload^[29]. Moreover miRNA-155 directly targets endothelial nitric oxide synthase (eNOS) and the type 1 receptor of Angiotensin II (AT1R), primordial targets that regulate the tonus of vascular smooth muscle cells (VSMC), and hence the peripheral cause of pressure

overload on the heart^[29-32].

With regard to ET and CH, Fernandes *et al.*^[5] have shown that swimming training was able to increase miRNA-27a and 27b [targeting angiotensin-converting enzyme (ACE)] and to decrease miRNA-143 [(targeting angiotensin-converting enzyme 2 (ACE2)] in the heart of rats. The CH induced by ET involves the regulation of miRNAs related to increased AT1R expression without the participation of Angiotensin II. Parallel to this, the increase in ACE2, Angiotensin (1-7) and type 2 receptor of Angiotensin II in the heart has also suggested that miRNAs were involved in upregulation of the non-classic renin-angiotensin aldosterone system (RAAS), counteracting the classic cardiac RAAS in physiological CH^[5]. Thus, it is plausible to suggest a relationship between ET and CH through the regulation of several targets by miRNA-155, -27a, -27b, -143.

A hallmark related to hypertension and pathological CH is the reactivation of a set of fetal cardiac genes, which are repressed postnatally and replaced by the expression of adult genes. These genes include atrial natriuretic peptide/B-type natriuretic peptide, skeletal α -actin, and β -myosin heavy chain (β MHC). The causes and consequences of fetal gene expression in the adult heart have not been completely elucidated, but is known that chronic stress on the heart, such as hypertension, increases β MHC (slow ATPase activity) and decreases α MHC (fast ATPase activity), which has been implicated in impaired cardiac function^[13,33,34]. It is well known that in cardiovascular disease, the expression of β MHC increase while α MHC decreases and that ET is able to reverse these abnormalities in rats^[20,33,34].

The miRNAs -208a, -208b, and -499 are called “*myomiRNAs*”, which regulate the expression of slow myosin, playing an important role in the control of cardiac disease progression^[35-38]. The inhibition of miRNA-208a with Locked Nucleic Acid-Modified Anti-miRNA-208a (LNA-antimiRNA-208a) induced reversion in MHC switching during heart failure in hypertensive rats, reduced deleterious cardiac remodeling, and prevented the deterioration of cardiac function and lethality in rats^[39]. In another study, the circulating miRNA-16, -19b, -20b, -93, -106b, -223, and -423-5p were equally reversed by both LNA-antimiRNA-208a and captopril therapy, and the results were correlated with the changes in β MHC expression in the time course of hypertension or therapy in rats^[40]. With regard to ET, recent studies performed in our laboratory showed that ET decreases cardiac miRNA-208a expression in healthy Wistar and obese Zucker rats, induces upregulation of targets as THRAP-1, Pur β and Sox6, and improves the balance between the β MHC and α MHC gene expression^[41,42]. Thus, miRNA-208a is another pathological miRNA naturally reversed by ET, and its downregulation is involved in the increase in several targets that constitute a gene program to improve the contractile efficiency of the heart^[35]. Regarding circulating miRNAs, the miRNA-208a and -499 also reflect cardiovascular damage and a poor prognosis in patients with viral myocarditis, acute myocardial infarction, hyperten-

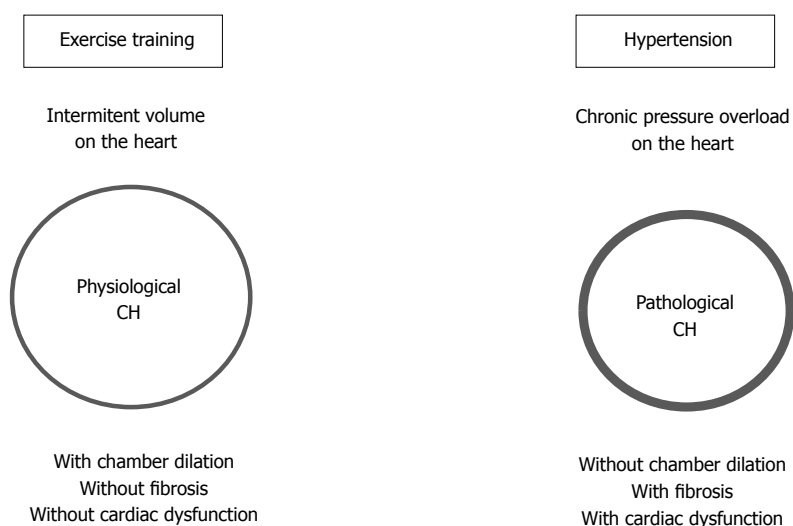


Figure 1 Effects of aerobic exercise training on the cardiac miRNAs in hypertension. CH: Cardiac hypertrophy; COL I : Collagen 1; COL III: Collagen 3; ACE: Angiotensin-converting enzyme; ACE2: Angiotensin-converting enzyme 2; THRAP-1: Thyroid hormone-associated protein 1; Purβ: Purine-rich element binding protein B; α-MHC: α-Myosin heavy chain; β-MHC: β-Myosin heavy chain.

Effect of exercise training	Targets	Phenotype	Effect of hypertension
↑ miRNA-29c	COL I ; COL III	Fibrosis	miRNA-29c ↓
↑ miRNA-27a ; miRNA-27b	ACE	Vascular resistance	miRNA-27a; ↓ miRNA-27b
↓ miRNA-143	ACE 2	Vasodilation	miRNA-143 ↑
↓ miRNA-208a	THRAP-1; Purβ; Sox6	Balance between α/β-MHC expression	miRNA-208a ↑

sion or diastolic dysfunction^[43].

Hypertension induces cardiac fibrosis. The aberrant expression of matrix extracellular proteins is determinant in the differentiation between pathological and physiological CH^[20,44]. There are also several miRNAs involved in the fibrotic response to stress and ischemic stimulus: the miRNA-21, -24 family, -29 family, -101, -206, -132, -214, involved in fibroblast survival, growth and differentiation or dysfunction of extracellular matrix^[44]. The downregulation of miRNA-29b induces upregulation of several extracellular matrix genes in the heart during pathological CH, including collagens and elastin^[45]. In addition, the miRNA-29c has recently been implicated in the immunopathogenesis of atrial fibrillation, showing a relationship between cardiac arrhythmia and abnormalities in miRNA-29c expression^[46]. ET is able to increase cardiac miRNA-29c expression, and a study conducted by Soci *et al*^[20] has shown that swimming training increased miRNA-29c, and downregulated collagens I and III in the CH of rats. Thus, the future perspective related to fibrosis, immune system and cardiac automatism points to an integrative and regulatory role of miRNAs in the fibrotic response of the heart, requiring further investigations about miRNAs and its mRNA targets involved in the processes that regulate diastolic function, cardiac automatism and hypertension. Another interesting miRNA that plays an important role in the dysfunction of cardiovascular system is the miRNA-34a, which is induced in the ageing heart. The inhibition of this miRNA reduces fibrosis following the acute myocardial infarction and improves recovery of myocardial function^[47]. In this way, studies linking this miRNA, cardiac fibrosis, and ET will contribute to the knowledge and treatment of cardiovascular dysfunctions

in the future.

According to the studies conducted by our laboratory, ET is able to reverse or prevent pathological processes involved in hypertension^[5,20,24,25,48], but further studies are needed, and will be performed to investigate the relations between ET, hypertension, and CH in the perspective of miRNAs.

The Figure 1 summarizes the effects of ET or hypertension on some cardiac miRNAs.

EFFECTS ON THE VASCULAR SYSTEM

Macro- and microcirculation interact in the vascular system, and their changes contribute to end-organ damage in hypertension^[49]. However, these two vessel types must be discussed separately because they are differently regulated^[50].

Macrocirculation

Macrocirculation comprises large arteries such as the brachial, radial, femoral, aorta, epicardial arteries, and others vessels with the purpose of supplying blood from heart to peripheral tissues, and also performs the function of transforming the pulsatile flow into a steady flow necessary to supply oxygen to the tissues. In order to do this, good arterial compliance and distensibility are required, but in hypertension these properties of the arteries are affected^[51].

In hypertension, the structure, mechanical behavior and function of vessels are affected, with a reduction in lumen diameter and thickening of the tunica media (structural change), increased vascular stiffness (mechanical change), and impaired NO-dependent vasodilation

(functional change)^[51]. The direct relationship between hypertension and thickening of large vessels is due to an adaptive response of VSMC to increased internal arterial radius secondary to increased wall tensile stress imposed by pulsatile blood flow. Thus, VSMC become hypertrophied and change from the contractile phenotype to a proliferating phenotype. In addition, there is an increased collagen content in vessels, which contributes to increases in arterial thickness^[49]. Furthermore, hypertension promotes decreased endothelial NO production, which leads to more collagen expression and VSMC growth, affecting the vascular thickening and stiffness^[52]. In fact, NO is able to inhibit the expression of collagen, and lack of NO may induce excessive proliferation of VSMC^[53]. Thus, ET can act positively to prevent or reverse these vascular changes induced by hypertension, such as hypertrophic remodeling that may be caused by loss of mitogenic quiescence of VSMC, resulting in their proliferation and establishment of a hypertrophied phenotype^[53,54]. ET may also act on the vascular stiffness caused by high expression of collagen, which can be induced by hyperactivity of the renin-angiotensin system, and may also act on the impaired endothelium-dependent vasodilation, improving the NO availability^[51].

A large body of evidence has indicated that ET exerts positive effects on preventing or reversing the structural, mechanical, and functional vascular changes in hypertension. Indeed, Moraes-Teixeira *et al.*^[55] have shown that the effects of treadmill ET (1 h/d, 5 d/wk, 20 wk) were able to decrease the circumferential wall tension and intima-media thickness in the aorta of exercised spontaneous hypertensive rats (SHR) compared with non-exercised animals, without significant differences in the lumen diameter among the studied groups at the end of protocol. Moreover, treadmill exercise increased the percentage of elastic fibers; percentage of eNOS density in the aortic wall, and decreased blood pressure in exercised SHR compared with their non-exercised controls. These results were interesting, because they showed that ET was able to prevent or reverse the capacity of hypertension to modulate the thickening and stiffening of large vessels in rats. Furthermore, Guimarães *et al.*^[56] assessing arterial stiffness by carotid-femoral pulse wave velocity in hypertensive patients has shown that interval ET (16 wk of training) decreased arterial stiffness in trained subjects. In addition, aerobic ET (30 min, 3 times/wk, 4 wk) was able to reduced arterial stiffness in young men with a family history of hypertension^[57]. Jordão *et al.*^[58], have shown that treadmill exercise was able to reduce the mRNA expression of collagen I and III; prevent rupture of the internal elastic lamina, and improve the orientation of VSMC in the aorta of trained SHR compared with non-trained animals. Furthermore, endurance training attenuated the oxidative stress in the aorta of SHR, showing a possible suppressive effect of the exercise on the development of arteriosclerosis^[59], and was able to increase endothelium-dependent relaxation through NO pathways in the aorta of SHR^[60].

Microcirculation

Microcirculation is a network of vessels that includes the smallest arteries, arterioles, capillaries and venules which, by definition, have an inherent physiological characteristic of responding to increasing pressure by a myogenic reduction in lumen diameter, rather than a definition based on the vessel diameter and structure. Microcirculation has the function of optimizing nutrient and oxygen supply within the tissue in response to variations in demand; avoiding potential fluctuations in the pressure at the level of the capillaries, and determining the overall peripheral resistance^[49,61].

In hypertension, the mechanisms regulating vasomotor tone may be abnormal, leading to altered vascular function; and structural and mechanical alterations may also occur, such as an increased wall-to-lumen ratio and arterial stiffness. Furthermore, the rarefaction of arterioles and capillaries affecting the microvascular network has been observed in hypertension, and at first, it seems to be a functional change that involves the constriction of microvessels to the point of nonperfusion, and the second change is structural, when the nonperfused vessels may disappear. It is important to note that these factors will contribute differently in each vascular bed, and may vary between models of hypertension^[49,50,61]. Central to these alterations there is impaired NO availability, secondary to oxidative stress, mainly due to the increased production of reactive oxygen species and reduced antioxidant capacity, as well as increased cyclooxygenase-derived contractile products^[49,50,62-66].

Microvascular damage is a predictor of long-term adverse cardiovascular prognosis. Endothelial dysfunction has been considered an independent predictor of adverse cardiovascular events, providing a better predictive value of future cardiovascular events than each traditional risk factor alone identified in the Framingham study^[67]. Moreover, the abnormal artery structure and the arterial stiffness of small vessels are predictors of later cardiovascular events and have prognostic implications^[68-71]. As regards rarefaction, further prospective study is needed to determine whether it presents a clinically relevant predictive value, however it is important to note that microvascular rarefaction will reduce oxygen delivery resulting in ischemia, which may be responsible for much of the end-organ damage associated with hypertension^[49,61].

Given the central role that all these alterations play in vascular biology, it seems attractive to consider the relevance of therapeutic improvement in function, structure and mechanical alterations in hypertension. Thus, non-pharmacological approaches, such as ET, are able to improve blood pressure control and vascular alterations in hypertension. It is well established that ET decreases blood pressure^[24], and although endurance training, dynamic resistance training and combined training were associated with decreases in blood pressure, until clearer evidence emerges, it may be prudent to prescribe endurance training for the hypertensive individual^[24]. In accordance with Cornelissen *et al.*^[72], aerobic endurance training

decreases blood pressure through a reduction in vascular resistance.

Regular ET improves endothelial function in hypertensive patients as well as in animal models of hypertension^[73,74]. Although to a lesser extent, some recent studies have also confirmed the beneficial effects of continuous aerobic ET on the endothelial function of small arteries, such as in arteries of gastrocnemius muscle from rats with chronic NO synthase inhibition^[75]; in mesenteric resistance arteries and small coronary arteries from SHR^[15]; and in resistance arteries from young pre-hypertensive patients^[76]. Emerging evidence has increasingly demonstrated that diverse beneficial effects induced by ET in hypertension are mediated, at least in part, by reversing oxidant stress^[77]. In fact, in small arteries and arterioles the reduced oxidative stress significantly contributes to endothelium-dependent vasodilation that has been enhanced by ET^[15,78].

In addition to the functional improvement, structural and mechanical changes are also mediated by exercise in hypertension. Recently, we demonstrated that aerobic treadmill exercise reverts the increased arterial stiffness of both mesenteric resistance and small coronary arteries, mediated by changes in the extracellular matrix^[15], although it did not modify the increased wall-to-lumen ratio of these arteries in hypertension. However, the vascular remodeling induced by ET may be dependent on the vascular bed studied, and thus either improvement^[79,80] or no effects^[15,79,80] already have been observed in microcirculation in hypertension. In addition to structural changes, aerobic ET corrects capillary rarefaction in hypertension^[7,79]. Indeed, a balance between angiogenic and apoptotic factors to prevent microvascular abnormalities in hypertension has been observed as an effect of ET^[7]. In addition, the decrease in oxidative stress induced by ET in SHR seems to be associated with the normalization of the reduced number of endothelial progenitor cells in a vascular endothelial growth factor (VEGF)/eNOS-dependent pathway, thus promoting a peripheral revascularization induced by aerobic ET^[11].

Although many vascular effects of ET have been established in hypertension, little is known in the literature about their beneficial effects on miRNAs of the small and large vessels. However, recently new approaches in ET have highlighted the key role of miRNAs in the modulation of hypertension.

The miRNAs are involved in all biological processes, including cellular proliferation, differentiation, cellular migration and apoptosis, and their deregulation often results in the development of cardiovascular diseases. As there is high expression of miRNAs in the vascular system, growing evidence suggests that miRNAs may be important in the development of endothelial dysfunction, vascular remodeling and reduced angiogenic capacity, features that are frequently observed in the pathogenesis of hypertension^[2,81,82]. More specifically, the phenotypes of VSMC and endothelial cells, as well as the inflammatory activation of macrophages is regulated by miRNAs, which may promote the structural changes that lead to

vascular remodeling^[83]. VSMC maintains remarkable plasticity, able to react to various forms of vascular stress or injury by switching from the contractile phenotype to a proliferating and synthetic phenotype^[2].

Studies have associated hypertension and alterations in the expression of miRNAs with the angiogenic process, endothelial dysfunction, changes in the RAAS, and in the phenotype of VSMC^[2], but as regards the role of ET, there is almost nothing in the literature showing a direct connection between the modulation of miRNAs and vascular changes in hypertension. For example, nothing has yet been shown in the literature relating hypertension and the effects of exercise to the miRNAs existing in aorta. However, considering the atherosclerosis, Wu *et al*^[84] investigated the effects of treadmill ET for a period of 12 wk, 5 times per week, 60 min/d, on the aorta of male ApoE null C57BL/6J mice with atherosclerosis, which were fed a high-fat diet. The authors showed that ET significantly decreased the angiotensin II and endothelin 1, and prevented the formation of plaques and foam cells in comparison with the control group, followed by decreased expression of miRNA-155, and increased expression of miRNA-146a and miRNA-126 in the aorta of the trained mice, with more pronounced changes in the groups treated with Simvastatin. The miRNA-146a interacts with the 3'-UTR of the tumor receptor-associated factor 6 (TRAF6) gene, negatively impacting the toll-like receptor 4 (TLR4)-TRAF6 signaling, and then reduces the inflammatory response in atherosclerosis^[85]. The decrease in miRNA-155 expression is an essential factor for increasing eNOS expression and NO production, because eNOS is directly targeted by this miRNA^[31,86]. In atherosclerosis, miRNA-155 is drastically upregulated^[31], because inflammation factors increase miRNA-155 *via* activation of nuclear factor (NF)- κ B, activator protein-1, and Rho kinase. The miRNA-155, together with yet unidentified cytosolic RNA-binding proteins, bind to the eNOS mRNA 3'-UTR and destabilize eNOS mRNA, resulting in decreased eNOS and NO production. However, anti-miRNA-155, statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) and other Rho kinase inhibitors prevent the increase in miRNA-155^[86], and then maintain the eNOS and NO production, or possibly increase their production. However, in hypertension, could ET diminish miRNA-155 expression in vessels? To our knowledge, at present there are no studies to answer this question. Nevertheless it is known that exercise can modulate the miRNA-155 expression in atherosclerotic vessels.

The study of Wu *et al*^[84] has also shown that ET was able to increase the miRNA-126 expression in the aorta of the studied mice. The miRNA-126 suppresses the cell adhesion molecule expression and negatively regulates the endothelial receptor of α 4 β 1 integrin, thereby interfering with adhesion of leucocytes to the endothelium^[87,88], as well as enhancing angiogenesis^[87]. In agreement with Harris *et al*^[87], miRNA-126 is expressed in endothelial cells, but not in VSMC, and miRNA-126 in other tissues might simply reflect the vascularity of the organ. Target deletion of miRNA-126 in mice promotes hemorrhaging, loss of

Table 1 Vascular effects of hypertension and exercise training in macro- and microcirculation

Hypertension	Exercise training	Ref.
Hypertrophy of VSMC	Decreased intima-media thickness	[49,52,55-57]
Excessive proliferation of VSMC	Improved orientation of VSMC	[49,53]
Increased collagen content	Decreased mRNA collagen expression	[49,51,58]
Decreased availability endothelial NO	Increased endothelial NO production	[49,51,55]
Increased endothelium-dependent contract factors	Increased endothelium-dependent relaxation	[59,75]
Increased ROS production	Increased antioxidant capacity	[49,50,59,62-66]
Increased overall peripheral resistance	Reduced vascular resistance	[24, 49,50]
Rarefaction of arterioles and capillaries	Increased peripheral revascularization	[7,49,50,61,79]

VSMC: Vascular smooth muscle cells; NO: Nitric oxide; ROS: Reactive oxygen species.

Table 2 Some miRNAs associated with hypertension that has potential to be regulated by exercise training

miRNAs	Targets	miRNA function	Ref.
miRNA-16	VEGF	Control of angiogenesis and vascular integrity	[7]
miRNA-21	PTEN; Bcl-2	Involved in nitric oxide production; apoptosis	[2,7,80,82]
miRNA-24	Trb-3	Mediator of contractile phenotype in VSMC	[81-83]
miRNA-92a	Integrin α 5; eNOS	Involved in the regulation of endothelial function	[88]
miRNA-126	VCAM-1; PI3KR2; Spred1	Suppress cell adhesion molecule; Proangiogenic	[48,85,87,88]
miRNA-143/145	KLF4; KLF5	Involved in the plasticity of VSMC	[81]
miRNA-146a	TRAF6; KLF4	Involved in inflammatory response	[85]
miRNA-155	eNOS; AT1R	eNOS expression and NO production	[85]
miRNA-221/222	P27Kip1	Involved in the proliferation of VSMC	[81]

VEGF: Vascular endothelial growth factor; PTEN: Phosphatase and tensin homolog; Bcl-2: B-cell CLL/lymphoma 2; Trb-3: Tribbles-like protein-3; eNOS: Endothelial nitric oxide synthase; NO: Nitric oxide; VECAM1: Vascular cell adhesion molecule 1; PI3KR2: Phosphatidylinositol 3-kinase regulatory sub-unit beta; Spred1: Sprouty-related; EVH1: Domain-containing protein-1; KLF4: Krüppel-like factor 4; KLF5: Krüppel-like factor 5; TRAF6: TNF receptor-associated factor 6; AT1R: Type 1 receptor of angiotensin II; P27Kip1: Cyclin-dependent kinase inhibitor 1B.

vascular integrity and defects in endothelial cell proliferation, migration and angiogenesis^[89]. The miRNA-126 is one of the most abundant miRNA in endothelial cells, and plays an anti-atherogenic role by enhancing endothelial repair^[2,82,84].

Another miRNA that deserves to be remembered is the miRNA-34a because its overexpression has been associated with senescence of endothelial cells. This miRNA targets SIRT1 (sirtuin 1) in the endothelial cells^[90] and may serve to mediate the effect of aging upon the vasculature^[91]. The inhibition of SIRT1 impairs the eNOS metabolism in the endothelial cells *via* SIRT1/eNOS axis^[92,93], allowing us to suppose that this miRNA may be related with development or progression of atherosclerosis or hypertension. However, such as in heart, the literature has not shown studies linking the miRNA-34a, vascular cells, and ET.

In addition to the above mentioned data, other miRNAs are able to participate in the vascular changes. In this case, the miRNA-143/145 cluster is related to the phenotype plasticity of VSMC, and together with miRNA-21 and miRNA-24, they are involved in the differentiation and proliferation of these cells. Thus, several studies have shown that they are downregulated in injured vessels^[2,81-83]. The miRNA-21 is involved in vascular remodeling that affects both VSMC and endothelial cells. The overexpression of miRNA-21 induces a synthetic VSMC phenotype, as observed after vascular injury, and it is also a critical miRNA for angiogenesis^[2,81,82]. The miR-

NA-221/222 have an ambiguous response after vascular injury, enhancing the proliferation of VSMC, whereas it may be atheroprotective in endothelial cells^[81,83]. The miRNA-221/222 may also be involved in the control of eNOS expression^[94]. The miRNA-92a is a negative regulator of endothelial function, and its overexpression represses *eNOS* gene expression and angiogenesis^[88]. The abovementioned miRNAs have been found to be related to hypertension. B tkai *et al*^[2] and Synetos *et al*^[95] provided an overview of the role of miRNAs in the development and consequences of hypertension.

Recent data from our research group has shown the effects of ET on vascular miRNAs involved in hypertension. Our study reveals some of the molecular mechanisms of ET in physiological revascularization observed in hypertensive rats. Swimming ET restored the balance between injury and repair in the vascular process collaborating with the regression of hypertension, and remarkably, restoring normal expression of skeletal muscle microcirculation miRNA-16, -21, and -126^[7]. This alteration occurred parallel with normalization of VEGF, eNOS, and PI3KR2 levels, as well as the proapoptotic (Bad) and antiapoptotic (Bcl-2, Bcl-x, and p-Bad^{ser112}:Bad ratio) mediators, indicating that balance between angiogenic and apoptotic factors may prevent microvascular abnormalities in hypertensive rats^[7].

The study of miRNAs may generate hypotheses about the mechanisms by which exercise affects the pathophysiology of hypertension. ET probably causes al-

terations in many of the miRNAs that are deregulated in the vascular system. Thus, a promising tool emerges for the treatment and expansion of knowledge about hypertension.

Effects of hypertension and ET in the vascular system are summarized in Table 1, and some miRNAs, its target, and miRNA function are in the Table 2.

EFFECT ON THE SKELETAL MUSCLE

Microvascular abnormalities, such as reduction in blood flow and microvascular rarefaction, are clear evidence of disturbance of the angiogenic process related to changes in the muscle fiber profile in hypertension^[61,96,97].

It is interesting to note that studies have shown that ET-induced blood pressure reduction in SHR was correlated with both normalization of arterial wall-to-lumen ratio and a great increase in capillary-to-fiber ratio in skeletal muscle. Indeed, evidences have shown that ET improves both endothelial function and muscle fiber profile, counteracts microvascular rarefaction and decreases blood pressure in hypertension^[7,11,79,80,98].

It is known that the angiogenesis represents a primary adaptive response of the skeletal muscle to aerobic ET, hence contributing to the improvement in muscular aerobic capacity (oxygen transportation, provision and extraction)^[79]. On the other hand, many conditions, such as cardiovascular disease (CVD) risk factors, lead to alteration in the capillary support of skeletal muscles, and may consequently, impair the offer of oxygen and nutrients, which is related to alteration in the distribution of the skeletal muscle fiber types towards an increase in type II fibers. As yet, little is known about the origin of the transition from type I fibers to type II in the soleus muscle of SHR; however, studies have shown that it is related to capillary rarefaction followed by alterations in metabolic properties^[96,99].

Studies have shown that when there is a transition between the types of fibers of the skeletal muscle, the different morphological properties of the muscular fiber are changed in the following manner: the capillary density and activities of the energy metabolism enzymes are altered at an early stage during the transition, and precede the change in myofibrillar ATPase activity and the contractile characteristics of the muscle^[100].

In mammals, the skeletal muscle fibers are usually classified as type I and type II fiber, according to the different activities of the myosin ATPase after pre-incubation at different pHs, and the type II fibers can be sub-classified into II A, II X/D and II B. The type II fibers are characterized as being fast twitch with predominance of glycolytic metabolism, while the type I fibers are slow twitch with predominance of oxidative metabolism^[96].

Evidences in the literature have shown that the skeletal muscle of hypertensive individuals, and of SHR, contains a higher percentage of type II fast twitch, glycolytic fibers compared with their normotensive controls^[7,96,100,101]. It is interesting that the results obtained in the analysis of the composition of the fiber types of the

soleus skeletal muscle (which presents an average of 90% of type I fibers and 10% of type II fibers), performed both by histochemical myosin ATPase reaction and SDS-PAGE gel electrophoresis for detection of MHC for each type of fiber, were positively correlated regardless of the technique applied^[101]. According to Bortolotto *et al.*^[101] the main result obtained in their study was that in all stages of hypertension (4, 16 and 24 wk), the soleus muscle of SHR presented a higher proportion of type II fibers than the soleus muscle of Wistar Kyoto rats (WKY), as well as hybrid fibers, those that contain two types of MHC in the same muscle fiber isolate, in the case of SHR, a higher proportion of II A + II X hybrid fibers. The presence of a higher proportion of hybrid fibers is an indication of the transition of muscle fiber type in the muscle under consideration.

Some studies have associated the effects of ET with pharmacological treatment. Minami *et al.*^[102] showed the effects of ET either associated with treatment with perindopril (ACE inhibitor) or without it, on the capillarity and fiber types in the soleus muscle of SHR. The authors observed that chronic treatment with perindopril increased the exercise capacity in untrained animals; however, this effect was not synergic to the exercise capacity acquired as a result of ET alone. Whereas, the treatment with perindopril associated with ET promoted adaptive alterations in the soleus muscle, such as increase in capillary density and percentage of type I fibers^[102]. Although no alteration in the composition of types of fiber was observed in the trained SHR and SHR treated with perindopril groups when compared with the sedentary SHR group, the authors observed higher capillarization in these groups, which may be attributed to the improvement in exercise capacity. A more recently study from the same group showed that pharmacological treatment with a calcium channel blocker (azelnidipine), or a type I angiotensin I receptor antagonist (olmesartan) or even the ET significantly increased capillary density and percentage of type I fibers in the soleus muscle of SHR^[103]. Although the results in the literature are still controversial with respect to the alterations in proportion of the types of fiber in response to ET, it was also not possible to observe the comparison between the profile of the types of fiber in the trained SHR group compared with its normotensive control WKY, with the aim of checking normalization with the fiber type composition.

Recently, Fernandes *et al.*^[7] for the first time, showed evidence that aerobic ET corrected the alteration in the composition of fiber types in the soleus muscle of SHR when compared with WKY. This result is probably linked to the increased capillarization and citrate synthase activity observed with ET, since these adaptations are related to changes in fiber type in the skeletal muscle. Altogether, these ET-induced adaptations contribute to the increase in oxygen consumption and exercise tolerance, and the decrease in BP levels observed in the trained hypertensive group.

Although studies have reported change in the profile of skeletal muscle fibers in hypertension, none of them

observed change in muscle mass in hypertensive rats up to 24 wk of age^[7,96,100,101,104]. It is interesting that Carvalho *et al.*^[105] determined the soleus muscle changes in the expression of MHC isoforms, diameter of fiber types and muscle atrophy during the transition of ventricular hypertrophy to heart failure induced by aortic stenosis. The animals developed a myopathy in the soleus muscle, characterized by a decrease in the percentage of type I fibers and increased frequency of type IIa fibers, in cardiac hypertrophy (after 18 wk) and heart failure (after 28 wk). However, atrophy of type IIa fibers occurred only during heart failure.

Recently, for the first time in the literature, Damatto *et al.*^[106] reported changes in MHC isoforms and soleus muscle atrophy induced by heart failure in SHR. The setting of heart failure in SHR at 18 mo of age was observed and muscle disorders were associated with myogenic regulatory factors and expression of myostatin and follistatin.

Many studies have shown the beneficial effect of ET on muscle atrophy and correction of changes in fiber types in animals with heart failure due to various etiologies, such as myocardial infarction, and sympathetic hyperactivity^[99,107], however no study up to now has reported the effects of ET on these changes in animals with heart failure with the etiology of hypertension.

In spite of the important role of exercise in the prevention and treatment of hypertension, the mechanisms involved in these vascular and muscle changes are not fully understood. The analysis of miRNAs has made it possible to understand the development of various types of CVD, and the elucidation of these processes regulated by miRNAs and identification of new targets of miRNA in the pathogenesis of disease is a very valuable strategy for both prevention and treatment of hypertension.

Recent studies have revealed that myogenic transcription factors involved in differentiation and muscle contraction also activate the expression of a set of miRNAs with the function of “adjusting” the output of the transcription network, resulting in precise cellular responses to signals of development, physiology and pathology. The integration of these small RNAs into the muscle transcriptional program further expands the accuracy and complexity of the regulation of genes in muscle cells, since miRNAs are capable of regulating various mRNAs, and mRNAs can be targets of many miRNAs^[108-110].

The miRNAs-1, -133a-b, -206 and -208 are muscle-specific and have been studied thereby contributing to muscle development. It is interesting that these miRNAs provide up to 25% of miRNAs expressed in skeletal muscle; they are recognized by their control of the growth, differentiation and contractility of muscle^[111-116]. Additional miRNAs have been described; these regulate myoblast proliferation or differentiation, and include miRNAs-24, -26a, -27b, -125b, -148a, -181, -214 and -489^[116-119]. Curiously, high expression of miRNA-128a was found in skeletal muscle, and increased during myoblast differentiation, regulating target genes involved in insulin signaling, which include insulin receptor (Insr),

insulin receptor substrate 1 (Irs1) and phosphatidylinositol 3-kinases regulatory 1 (Pik3r1). In fact, Motohashi *et al.*^[116] showed that overexpression of miRNA-128a in myoblasts inhibited cell proliferation by targeting Irs1. In contrast, inhibition of miRNA-128a induced myotube maturation and myofiber hypertrophy *in vitro* and *in vivo*.

The miRNAs-1 and -133 are expressed in cardiac and skeletal muscle and they are transcriptionally regulated by myogenic differentiation factors, such as MyoD, myogenin, Mef2 and SRF (serum response factor)^[110-113]. The miRNA-1 promotes differentiation of cardiac and skeletal progenitor cells and exit from the cell cycle in mammals^[109], while the miRNA-133 inhibits differentiation, and maintains cells in a proliferative state^[111].

Increased expression of miRNA-1 in skeletal muscle of mice after 3 h of a single session of aerobic ET was observed by Safdar *et al.*^[120]. This increase was associated with a reduction in the expression of its target histone deacetylase 4 (HDAC4), a transcriptional repressor of muscle gene expression, and by the increase in myogenic differentiation factors such as MyoD, and thus would promote remodeling of the lesion caused by the training session^[113,120]. Conversely, the chronic effect of exercise led to a decrease in the expression of miRNA-1 associated with muscle hypertrophy in favor of the expression of important genes in muscle growth, such as c-Met, hepatocyte growth factor and Insulin-like growth factor 1 (IGF-1). IGF-1 is a potential target of miRNA-1, which could partly explain the hypertrophic phenotype during the initial responses resulting from ET overload^[121,122].

The miRNA-206 is the only miRNA specifically expressed in skeletal muscle, and its expression appears to be induced by MyoD and myogenin during myogenesis, promoting differentiation^[113,114,123]. HDAC4, PAX7, MET and Notch3 are some of the target *miRNA-206* genes related to the muscle differentiation process^[113,115,123].

These skeletal muscle miRNAs also appear to participate in muscle diseases including cardiac hypertrophy, heart failure and muscular dystrophy, such as Duchenne muscular dystrophy^[113,115,122,124].

Studies have reported that an intron of the α MHC (*Myh6*) gene encodes a miRNA -miRNA-208a, which is necessary to increase β MHC (*Myh7*) in the heart of adult animals in response to stress and hypothyroidism^[35]. Given that miRNA-208a and their host myosin, α MHC, are only expressed in the heart, these results raise interesting questions with respect to which other miRNAs could control the fiber type and gene program in skeletal muscle contractile proteins^[38].

van Rooij *et al.*^[38] showed the existence of two miRNAs in MHC genes. The β MHC gene encoding the miRNA-208b, which has an identical sequence to the seed miRNA-208a, and differs at only three nucleotides in the 3' region. A third member of this family is miRNA-499, encoded by the gene *Myh7b*, a little studied myosin that shares extensive homology with the β MHC gene. These two miRNAs are expressed in skeletal muscle, are related with an oxidative profile, such as in the soleus, and have a feature of type I fibers with predominance of β MHC.

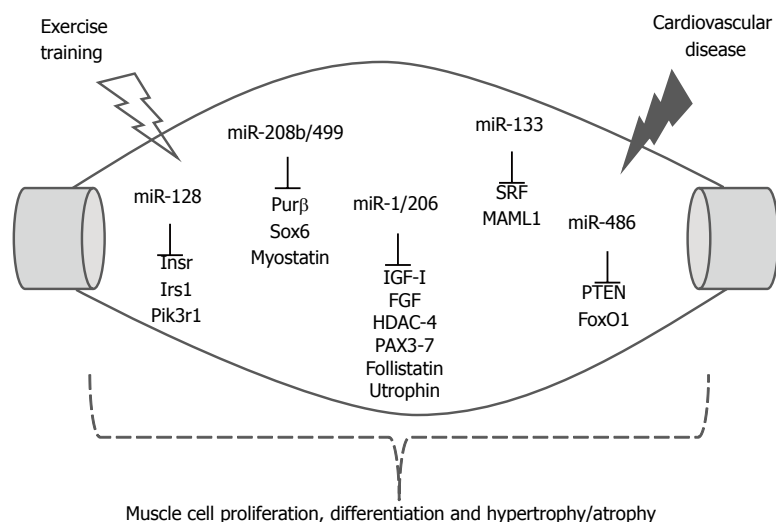


Figure 2 Skeletal muscle miRNAs and selected target genes regulating cell proliferation, differentiation and hypertrophy/atrophy by exercise training and cardiovascular diseases. The relationship between miRNAs and the mRNAs that encode proteins is shown. Insr: Insulin receptor; Irs1: Insulin receptor substrate 1; Pik3r1: Phosphatidylinositol 3-kinases regulatory 1; Purβ: Purine-rich element binding protein B; HDAC4: Histone deacetylase 4; IGF-1: Insulin-like growth factor 1; FGF: Fibroblast growth factor; SRF: Serum response factor; MAML1: Mastermind 1; PTEN: Phosphatase and tensin homolog; FoxO1: Forkhead box protein O1.

Interestingly, deletion of miRNA-208b and miRNA-499 did not alter the expression of another miRNA in the soleus muscle, and the analysis of fiber type showed little or no difference in the number of type I muscle fibers in any of the mutant animals compared with the wild type. However, in the generation of double knockout animals (dKO) for miRNAs-208b and -499 there was a substantial loss of type I muscle fibers in the soleus muscle of dKO. The loss of slow fibers in dKO mice was also evident from the reduction in protein and gene expression of β MHC, and a concomitant increase in the expression of isoforms of myosin fast type IIa and type II b and II x^[38].

Moreover, overexpression of miRNA-499 was sufficient to induce complete conversion of all fibers from soleus fast into slow fibers with a type I profile. Notably, when the animals were subjected to an exercise tolerance test on the treadmill, those with overexpression of miRNA-499 ran 50% more than the wild-type, indicating a higher aerobic endurance resulting from the reprogramming of muscle fibers with the induction of predominance of type I fibers, slow-twitch and oxidative metabolism. Moreover, the authors investigated the possible targets of these miRNAs related to the control of β MHC. The findings showed that the transcription factors Sox6 (a member of family Sox transcription factors) and Purβ are targets of miRNA-208b and -499 in skeletal muscle, and dKO animals have increased expression of both factors^[38]. Other studies have also shown that Sox6 and Purβ inhibit the expression of β MHC in skeletal muscle involved in changing the profile of muscle fibers^[125,126].

No studies have been conducted to evaluate the expression of these miRNAs and change in fiber type in CVD, in particular in hypertension. Knowing that in hypertension and CVD there is a change in muscle fiber profile, it would be appropriate to think that miRNAs-208b and -499 would participate in this change and that aerobic ET would be a strong candidate for the standardization of these parameters, since it is well known that ET increases the oxidative metabolism associated with a

predominance of type I fibers (Figure 2).

CONCLUSION

Considering that hypertension affects over one billion people across the world, that ET plays a key role as non-pharmacological therapy for hypertensive patients, and that genic therapies from miRNAs may represent new strategies in combating the development and/or progression of hypertension, is reasonable to go more deeply into studies to acquire more knowledge about which miRNAs are induced by ET and which are related to protection of the cardiovascular system. Most important, these studies may guide scientists in future gene therapies for the treatment of hypertension with specific miRNAs. Finally, complementing the above discussion is important to comment about circulating miRNAs that results of cellular damage and that have been presented as biomarkers of cardiovascular diseases^[127]. In this way, the circulating miRNA-1, -133a, and -208b were higher in patients with myocardial infarction in relation to patients who had unstable angina^[128], and the miRNA-499 was increased in individuals with acute myocardial infarction compared with patients without myocardial infarction^[129]. Also, patients with coronary artery disease or diabetes may presents reduced levels of circulating endothelial-enriched miRNAs, such as miRNA-126^[130]. Moreover, it was reported a linkage between circulating miRNAs, human cytomegalovirus, and essential hypertension^[131]. On the other hand, the literature has not presented studies linking circulating miRNAs, cardiovascular diseases, and ET. In this way, further studies need be realized. However, it is clear that circulating miRNAs will be used in the future also as biomarkers of the therapeutic efficacy of ET in the treatment of hypertension.

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Prehypertension: Underlying pathology and therapeutic options

Sulayma Albarwani, Sultan Al-Siyabi, Musbah O Tanira

Sulayma Albarwani, Sultan Al-Siyabi, Department of Physiology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat 123, Oman

Musbah O Tanira, Department of Pharmacology and Clinical Pharmacy, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat 123, Oman

Author contributions: Albarwani S, Al-Siyabi S and Tanira MO solely contributed to this paper.

Correspondence to: Dr. Sulayma Albarwani, Department of Physiology, College of Medicine and Health Sciences, Sultan Qaboos University, P.O.Box 35, Muscat 123, Oman. salbarwani@squ.edu.om

Telephone: +968-24141108 Fax: +968-24143514

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Abstract

Prehypertension (PHTN) is a global major health risk that subjects individuals to double the risk of cardiovascular disease (CVD) independent of progression to overt hypertension. Its prevalence rate varies considerably from country to country ranging between 21.9% and 52%. Many hypotheses are proposed to explain the underlying pathophysiology of PHTN. The most notable of these implicate the renin-angiotensin system (RAS) and vascular endothelium. However, other processes that involve reactive oxygen species, the inflammatory cytokines, prostaglandins and C-reactive protein as well as the autonomic and central nervous systems are also suggested. Drugs affecting RAS have been shown to produce beneficial effects in prehypertensives though such was not unequivocal. On the other hand, drugs such as β -adrenoceptor blocking agents were not shown to be useful. Leading clinical guidelines suggest using dietary and lifestyle modifications as a first line interventional strategy to curb the progress of PHTN; however, other clinically respected views call for using drugs. This review provides an overview of the poten-

tial pathophysiological processes associated with PHTN, abridges current intervention strategies and suggests investigating the value of using the "Polypill" in prehypertensive subjects to ascertain its potential in delaying (or preventing) CVD associated with raised blood pressure in the presence of other risk factors.

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Key words: Prehypertension; Renin-angiotensin system; Therapeutic lifestyle changes; Polypill

Core tip: There is a current debate over the ideal means of intervening in prehypertension. Since it is the cardiovascular risk that constitute the basis for intervention in both prehypertension and hypertension, the review discusses the following points, that: (1) categorizing blood pressure levels is based on mere 20 mmHg brackets; hence this doctrine may be re-visited to include other cardiovascular risk factors to categorize patients regardless of their blood pressure level; (2) investigating the therapeutic potential of intervening in all pathophysiological processes associated with prehypertension; and (3) ascertaining the therapeutic value of the "Polypill" in prehypertensives as means of primary prevention.

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SEARCH AND ARTICLE COLLECTION METHOD

Initially, PubMed and Scopus were searched using the

key words prehypertension, epidemiology, and treatment (as such or in the form of derived terms as appropriate) alone and in combination, were used to identify a set of primary articles. Other searches using pertinent key words were conducted as required; such was particularly utilized during search for pathophysiologically-related publications. For example, treatment + prehypertension led to renin-angiotensin system, which led to angiotensin converting enzyme system, angiotensin converting enzyme inhibitors, angiotensin receptor blockers and so on. To the resulted articles we added our own collection. Reviews were used as another source to include more articles. All searches were limited to English language.

The main aim of this review was to provide an overall understanding of the current status of the medical profession opinion on prehypertension and to map the so far recommended or suggested therapeutic strategies. It also reflected on other potential therapeutic options. The selection of cited references was made to serve that aim.

CONCEPTUAL OVERVIEW

Definitions

The concept of prehypertension (PHTN) was introduced in 1939 by Robinson and Brucer who were first to draw attention to the range of blood pressure (BP) between 120-139 mmHg (systolic) and 80-89 mmHg (diastolic) as being of value in determining clinically overt hypertension (HTN)^[1]. Almost three decades later, the same BP range was given the name “borderline hypertension”^[2], then the name changed to “high-normal blood pressure” in 1997^[3]. The name “prehypertension” was given in 2003^[4]. The nomenclature went further by some authors^[5] who categorized BP levels between 130-139/85-89 mmHg (the upper half of PHTN range) as “Stage 2” PHTN. Also, the upper half of the HTN range was given the name of “high normal” blood pressure by the European Society of Hypertension/the European Society of Cardiology^[6].

HTN is defined as a systolic/diastolic pressure level of $\geq 140/90$ mmHg^[7,8]. HTN is a major world health problem and is among the most prevalent chronic conditions with rates that reach up to 70% of adult population in some countries^[9] and is on the increase^[10]. HTN has been identified as the leading global risk factor for disease burden^[11] and it is considered to be the most important modifiable risk factor for coronary heart disease, stroke, congestive heart failure and end-stage renal failure^[12].

Salient issues pertaining to blood pressure and cardiovascular risk

Prior to start the discussion on the main topic of this review i.e prehypertension, there are a number of issues that are of significant value to this topic and they address the relationship of changes in BP and their correlation with cardiovascular (CV) morbidity and mortality. These are; the J-blood pressure curve concept; the central versus peripheral blood pressure relation with CV events; and the complex interaction of different antihypertensive

drugs on blood pressure and cardiac hemodynamics. A brief account of each of these follows.

The J-blood pressure curve concept and cardiovascular risk

The “J-curve” concept describes the shape of the relationship between BP and the risk of CV morbidity or mortality^[13]. Some authors consider the J-curve to be more correlated with diastolic blood pressure (DBP) than systolic blood pressure (SBP)^[14] since most of coronary blood flow occurs in diastole^[15]. Three pathophysiologic mechanisms have been proposed to explain the existence of a J-curve are: (1) low DBP could be an additional risk factor to coexisting or underlying poor health or chronic illness leading to increasing morbidity and mortality; (2) low DBP could be caused by an increased pulse pressure reflecting advanced vascular disease and stiffened large arteries; and (3) over-aggressive antihypertensive treatment could lead to too-low DBP and thus hypo-perfusion of the coronaries resulting in coronary events^[13].

The J-curve concept is in line with the thought that BP has a continuous relationship with CV events such as myocardial infarction, stroke, sudden death, heart failure and peripheral artery disease as well as of end-stage renal disease^[16-20] even at values such as 110-115 mmHg for SBP and 70-75 mmHg for DBP^[21,22].

Central systolic versus peripheral systolic blood pressure and cardiovascular events

In adults, peripheral SBP (pSBP) exceeds central (aortic) SBP (cSBP) by about 10 mmHg or more^[23]. This difference is greater in younger subjects, during exercise and is affected by drug therapy^[23]. Because cSBP and cPP are more closely related to the load on the heart and pulsatile stress on the coronary arteries than pSBP, they are suggested to be better predictors of CV events^[24,25]. Additionally, it may be highlighted that the heart, kidneys, and major arteries supplying the brain are exposed to aortic rather than peripheral pressure. Therefore, there is a rationale to believe that CV events may be more related to central rather than brachial pressure^[26].

The increase in central pressure from diastolic to systolic values is determined by the compliance of the aorta as well as the ventricular stroke volume. A high central pulse pressure (PP) is considered to be a marker of increased artery stiffness and represents a well-established independent predictor of CV morbidity and mortality^[27-29] in hypertensive individuals and even in those considered as having normal BP^[30]. PP significantly predicts major adverse CV events including unstable angina pectoris, myocardial infarction, coronary revascularization, stroke, or death^[31]. An independent correlation between aortic PP and coronary artery disease was established in men, along with age and hypercholesterolaemia^[32,33]. The late decrease in DBP after the age of 60, associated with a continual rise in SBP, is consistent with increased large artery stiffness. Higher SBP, if left untreated, may accelerate large artery stiffness and thus perpetuate a vicious cycle^[21].

Indeed, central pressure was found to be more (than peripheral pressure) correlated with indicators such as carotid intima-media thickness^[24,34,35] and left ventricular mass^[35-37]. Also, aortic pulse pressure was found to be significantly and independently correlated with angiographically determined coronary artery stenosis^[38] and more related to CV events than brachial pressure^[24,39-41] and responds differently to certain drugs^[25,42]. For example, it was found that the β -blocker, atenolol, is inferior to other major anti-hypertensive drug classes in preventing CV events. β -blockers exert differential effects on brachial *vs* central pressure which may help to explain the adverse findings with atenolol in outcome studies and provides support for the hypothesis that drugs which lower central pressure the most will be more effective^[43-48].

Antihypertensive drugs and cardiovascular events

The interaction of antihypertensive drugs on BP and coronary hemodynamics (and hence CV events) is complex. For example, not all antihypertensive drugs have similar effects on pulse pressure. Blockers of the renin-angiotensin system, calcium antagonists and diuretics improve arterial compliance and thus lower SBP more than DBP and therefore diminish pulse pressure. In contrast β -blockers, because they decrease heart rate, increase stroke volume would have a less favorable effect on pulse pressure than the other drugs. Yet, decreased heart rate may allow for more prolonged diastolic perfusion of the coronary vascular bed and *vice versa*; whereas, short-acting calcium antagonists and other arteriolar vasodilators (such as hydralazine, minoxidil) are prone to cause myocardial ischemia in susceptible patients^[49].

Antihypertensive drugs that reduce left ventricular hypertrophy (LVH) and hypertensive vascular disease are more effective over the long term in improving coronary flow reserve than drugs that have little or no effect. Thus, blockers of the renin-angiotensin system, calcium antagonists as well as the diuretics, have been shown to reduce LV hypertension^[50] and hypertensive vascular disease^[51-53] and improve arterial compliance^[54] better than β -blockers.

EPIDEMIOLOGY OF PHTN

Many studies in various countries were performed to determine the magnitude of the PHTN rate. These have revealed that PHTN prevalence is considerable and it varies widely from country to country. For example, prevalence rate averages at 21.9% in China^[55], at 32.8% in the Netherlands^[56], at 34% in Taiwan^[57], at 37% in the United States^[58], at 40% in Ghana^[59], at 48.2% in Oman^[60] and at 52% in Iran^[61]. Men^[57-59,61] and blacks^[62] are more likely to be affected than women or whites; respectively.

CARDIOVASCULAR RISK OF PHTN

PHTN is not only a caveat to develop overt HTN, but it is a major health risk on its own also. Prehypertensives were repeatedly reported to be subjected to approximate-

ly double the risk of CVD independent of progression to HTN^[58,63] in addition to other cardiovascular complications^[64-66].

PATHOPHYSIOLOGIC CHANGES ASSOCIATED WITH RAISED BP

This part of the review is intended to discuss briefly the significant pathophysiologic changes associated with the progressive increase in BP to provide the scientific premise for currently recommended interventions or another that is recommended by this review.

INVOLVEMENT OF THE RENIN-ANGIOTENSIN SYSTEM (RAS)

Effects of RAS on cardiovascular system in general

Angiotensin II, an active peptide of the RAS, causes increase in BP and enhances oxidation of the low-density lipoprotein *via* stimulation of its type 1 receptor (AT1)^[67,68]. It appears that it acts in this respect by inhibiting NAD(P)H oxidase-mediated oxygen synthesis and enhances antioxidant superoxide dismutase activity in the cardiovascular system and decreases nitric oxide (NO) bioavailability. The latter effect may be responsible, at least in part, for the beneficial effects of drugs inhibit RAS activity such as angiotensin converting enzyme inhibitors (ACEIs) or angiotensin II type 1 receptor blockers (ARBs) that may act, eventually, by enhancing NO availability^[69-71]. However, RAS blockade provides additional protective effect on cardiovascular function that cannot be solely explained by mere reduction of BP which is the action mediated by increasing NO availability^[72].

In this context, it may be added that angiotensin-converting enzyme 2 (ACE2) converts angiotensin I to angiotensin (Ang)-1-9, that can be converted by ACE to a shorter peptide, Ang-1-7, which has an intrinsic vasodilator activity^[73,74]. ACE2 have been described to be a potent negative regulator of RAS, counterbalancing the multiple functions of ACE, thus, it plays a protective role in the CV system and other organs^[75].

Also, chronic activation of the RAS was shown to underlie HTN, insulin resistance, cardiac and renal disease, and polycystic ovarian syndrome and it serves as a link between obesity and low-grade systematic inflammation^[76-80]. In addition, it is suggested that RAS contributes to the atherosclerotic process through angiotensin II, which acts as a proinflammatory mediator directly inducing atherosclerotic plaque development and heart remodeling and exacerbate endothelial dysfunction^[81,82]. On the other hand, blockade of RAS can offer protection from RAS-related metabolic diseases including diabetes^[83-88].

The statement by Demirci *et al*^[72] is further enforced by the observation that the ACE gene may be a determinant of serum ACE levels, but it does not appear to confer susceptibility to essential hypertension^[89], since there are many factors that influence the genetic make-up of

blood pressure^[90]. In addition, other environmental factors^[91] may be involved in determining BP. Therefore, the possibility of drugs interfering in the RAS to be additionally interfering with any of these other factors cannot be eliminated.

Effect of RAS on development of hypertension

The first report on the potential of early intervention to prevent HTN was in 1990^[92]. The authors showed that inhibiting RAS by captopril (an ACEI) for two weeks may intervene in the progression of HTN in young “pre-hypertensive” spontaneously hypertensive rats (SHRs). Later, other studies have shown that transient inhibition of the renin-angiotensin system from two weeks of age in SHRs, either with ACEIs or with ARBs, diminishes the increase in BP for up to 21 wk after cessation of treatment^[93]. While others reported that permanent treatment of SHRs from conception onwards with ACEIs completely prevented hypertension^[94,95].

The ARBs losartan was reported to have beneficial effect in humans^[96] and rats^[97,98] similar to that of captopril in SHRs, specifically, as shown by another study that transient use of losartan resulted in a long-lasting improvement of arterial contractility, an effect that was linked to endothelium-dependent vasodilatation^[92,98].

Paradoxically, other authors showed that decreased BP is accompanied by severe disruption of the normal vascular architecture of intrarenal arteries^[99]. These authors concluded that, apparently interference with RAS during a crucial stage of development in SHRs can initiate this disturbance and may cause intrarenal vascular smooth muscle hyperplasia, suggesting the involvement of another trophic factor that is inhibited by angiotensin II under physiologic conditions. Such led other workers^[72] to suggest that the efficacy of antihypertensive treatment is also influenced by age and the hypertensive stage of the investigate animals.

INVOLVEMENT OF VASCULAR ENDOTHELIUM

The association between RAS and endothelium-dependent pathways in PHTN was suggested by more than one observation. It was shown that dysfunctional NO synthesis in PHTN may be a source of oxygen free radicals or reactive oxygen species (ROS) which may be an additive factor to develop overt HTN^[100]. Jameson *et al.*^[101] reported that, endothelium-dependent relaxation of prehypertensive SHRs mesenteric arteries was impaired. Later, interleukin-1 β (which induces inducible NO Synthase) was shown to cause a lower production of NO and a reduced generation of cGMP in these animals^[102]. This observation was followed by demonstrating that lower NO level correlated with increased SBP in the same species^[103]. Such was related to impaired NO production alone^[104] or combined with an enhanced ROS activity which may contribute to progression of PHTN to HTN^[105]. All these effects in-

dicating that endothelial vasodilator capacity is impaired in PHTN^[106].

INVOLVEMENT OF REACTIVE OXYGEN SPECIES (ROS)

It is stated above that ROS may add to developing overt HTN. Therefore, it is not surprising that antioxidant deficiency has been long implicated in HTN pathogenesis^[107-109]; whereas, antioxidant treatment to reduce oxidative stress was shown to prevent development of HTN in SHRs^[110]. Many studies have demonstrated that enhanced production of plasma free radicals may impair the physiologic function of vascular endothelium^[111-113]. An action that may lead to increase in BP. Recently, the rationale for antioxidant trials in PHTN was reviewed by Nambiar *et al.*^[114].

INVOLVEMENT OF THE INFLAMMATORY PROCESS: PROSTAGLANDINS AND C-REACTIVE PROTEIN

Inflammation was also implicated in the development of HTN and in endothelial dysfunction either as a primary or a secondary event^[115]. Inflammation, indicated by C-reactive protein (C-RP) level, was used to predict HTN among PHTN subjects^[116,117]. In addition, prostaglandin E2 (an inflammatory cytokine) was particularly shown to enhance norepinephrine-pressor response in PHTN; an effect that was abolished by indomethacin (a prostaglandin synthesis inhibitor)^[118].

INVOLVEMENT OF THE AUTONOMIC NERVOUS SYSTEM

Eyal *et al.*^[119] suggested that α -adrenoceptors of SHRs are in a basic state of excitation even prior to the onset of overt HTN, *i.e.*, in PHTN. Prior to this observation, Fujimoto *et al.*^[120] demonstrated that β -adrenoceptor-mediated relaxation of arteries, in the same species, was diminished before and during development of HTN. The diminished relaxation may be because of defective hyperpolarization induced by these receptors^[121]. In the same rat species, both the M3 cholinergic- and P2y-mediated relaxation was not altered^[122] ruling out involvement of any other component of the autonomic nervous system apart from the sympathetic. However, β -blockers, compared to ACEIs, did not improve resistance arteries function after two years of use in human^[123,124].

The underlying mechanism for the sympathetic involvement was also indicated by the presence of sub-sensitive presynaptic α_2 -adrenoceptors which may lead to exaggerated norepinephrine secretion^[125], an effect that may have a causal relevance to development of HTN^[126]. Similarly, the β_2 -adrenoceptor-mediated facilitation of neurogenic pressor response was found to be enhanced in prehypertensive SHRs, which may contribute to devel-

opment of HTN^[127].

INVOLVEMENT OF CENTRAL MECHANISMS

An impaired baroreceptor control of vascular resistance was implicated in SHRs^[128] and such was thought to be a primary defect^[129]. In humans, baroreflex was not found to be altered, but plasma norepinephrine positively correlated to BP and associated with subsensitive α - and β -adrenoceptors^[130].

Another explanation of the sympathetic overactive state in PHTN/HTN was postulated by Kotchen *et al*^[131]. It is based on the observation that brain NO, as a neurotransmitter, reduces sympathetic output, and systemic angiotensin II activates NO-producing neurons. SHRs show higher gene expression of nNOS, probably, as a compensatory mechanism for increased BP. That hypothesis was supported by the finding that hypothalamic angiotensin II-sensitive neurons activity was greatly enhanced in PHTN^[132], and that the central component of the baroreflex was also impaired^[133].

INVOLVEMENT OF OTHER MECHANISMS

Other than RAS pathways should be investigated since not all the beneficial effects attributed to anti-RAS drugs (in HTN and PHTN) can be solely understandable by its mere reduction in BP. For example, RAS is activated by common comorbidity including type 2 diabetes, hyperinsulinemia and excess weight as well as it can be activated by a diet rich in carbohydrates and fats. Two clinical trials^[134,135] have shown that ACEIs decrease the risk of developing type 2 diabetes in patients with HYN and/or vascular disease.

THERAPEUTIC OPTIONS OF PREHYPERTENSION

Rationale for therapeutic intervention in PHTN

PHTN and HTN are associated with a number of factors such as increased age, male gender, increased C-RP level and waist circumference^[117,136]. These factors are positively correlated with the development of HTN^[117]. Yet and despite the clear relationship between HTN and PHTN, treating HTN is unequivocally accepted, but the debate over the use of the term PHTN itself as a clinical category^[137] or what type of intervention to be used in this case has not yet been concluded. The main reasons raising the thought of therapeutically intervening in prehypertensive subjects can be summarized as follows: (1) elevated systolic BP is the most important risk factor for cardiovascular, cerebrovascular, and renal disease^[4,16,138]; (2) there is a strong association of cardiovascular mortality risk with BP^[16]; (3) it is expected that many individuals with PHTN will, with time, become overt hypertensive patients^[139]; and (4) for normotensive population, it was calculated that SBP increases at an average rate of about

0.5 mmHg/year^[137].

Current suggested intervention strategies in PHTN

In principle, intervening in PHTN is a form of primary prevention, which can be enacted in more than one way. One is by the use of proven and safe drugs; another is by inducing individual behavioral changes. The latter is an attractive option because of its inherent “natural” appeal, perceived low cost, simplicity and safety though may not be sustainable.

Primary prevention strategies that are directed towards the individual necessitate screening all individuals in order to identify those who are over a certain “threshold”. That process is followed by subjecting individuals at risk to an appropriately “tailored” intervention to each of them which incurs high cost. In addition, risk prediction in primary prevention remains imprecise and may not reflect long-term risk^[140].

At the community level, primary prevention may be endorsed by passing health policies, encouraging beneficial cultural attitudes and/or imposing environmental changes. This approach is more likely to have a greater impact on individual's health^[141-144].

At present, it is agreed in principle, that prehypertensive subjects should be treated. However, there is a polarizing controversy on the means of intervention. Two main strategies are recommended; one is based on “Therapeutic Lifestyle Changes (TLCs)”^[4], and the second is based on using antihypertensive monodrug therapy^[5].

TLCS

Previous^[3] and current guidelines^[5] advocate specific lifestyle modifications for prehypertensives. The most recent recommendations (JNC7 report)^[4] are as follows: (1) maintain body mass index between 18.5 and 24.9 kg/m²; this is expected to reduce SBP by 5 to 20 mmHg for each 10-kg reduction in weight; (2) consume a diet rich in fruits and vegetables, as well as low-fat dairy products; this is expected to reduce SBP by 8 to 14 mmHg; (3) restrict sodium to no more than 6 g of table salt per day; this is expected to reduce SBP by 2 to 8 mmHg; (4) walk briskly at least 30 min per day or engage in other regular aerobic physical activity; this is expected to reduce SBP by 4 to 9 mmHg; and (5) reduce alcohol consumption; this reduces SBP by 2 to 4 mmHg.

Evidence for therapeutic effectiveness of lifestyle modifications

The JNC 7 lifestyle changes are focused on weight loss, dietary restriction and exercise, which were supported by abundant clinical evidence. For example, weight loss^[145], and salt restriction^[146] have been shown to improve PHTN. Maintaining a body mass index between 18.5 and 24.9 kg/m² is expected to reduce SBP by 5 to 20 mmHg for each 10-kg reduction in weight^[16].

Weight loss has been shown to be the most effective lifestyle modification strategy for prevention of hypertension^[147]. Reductions in BP occur even without attain-

ment of normal body mass index. In a meta-analysis of 25 randomized, controlled trials, weight loss of 1 kg was associated with approximately 1 mmHg reduction in SBP and DBP in individuals with HTN^[148]. Addition of antihypertensive medication has been shown to have an effect on BP reduction that is additive to that achieved by weight loss alone^[148,149]. However, it has been shown that the type of medication prescribed may decrease the ability of the patient to lose weight^[147].

Dietary pattern changes, in general^[150] or specifically prescribed such as the Dietary Approaches to stop hypertension (DASH) plan^[151,152] which uses a diet rich in fruits, vegetables, legumes, nuts, and low-fat dietary products and low in saturated fats, induced a significant lowering of BP. Adhering to the DASH diet can reduce BP by 8-14 mmHg, an effect that was augmented even further when dietary sodium was restricted. The OmniHeart Collaborative Research Group study^[153] in which the DASH diet was modified to provide more protein and unsaturated fat and less carbohydrate, impressive reductions of BP were also achieved. The TOHP trial^[147], in a substudy of the DASH trial also showed that by reducing sodium intake to less than 100 mmol in your daily diet, in addition to dietary changes provided greater benefit than either approach alone^[154].

Similarly, there is ample evidence that exercise, independent of weight loss, decreases BP^[154-157]. A number of clinical trials demonstrated that increased physical activity can lower BP independent of any effect on body weight, although this finding is not universal^[158-160]. However, two meta-analyses concluded clearly that physical activity independently lowers BP^[161,162]. In one of these meta-analyses, 27 of 50 studies reported results in nonhypertensive subgroups, which presumably include a large proportion of participants with PHTN^[162]. Exercise alone has been associated with a 30% reduction in cardiac risk, making it similar to statin and antihypertensive interventions^[163-166]. Hence, a number of studies have been performed to examine the effects of aerobic and/or resistance exercise on BP in hypertensive, prehypertensive, and normotensive groups, and a recent review has examined the relevant findings^[167].

Nevertheless, some trials have shown that TLCs to have a modest and unsustainable impact to reduce CVD events when tested in large, long-term trials^[152,168]. This observation has been challenged by the PREMIER trial^[169] which studied the combined effects of diet, physical activity, and weight reduction in three groups of prehypertensive and hypertensive subjects over an 18-mo period. Although all three groups demonstrated significant reductions in BP in both prehypertensive and hypertensive subjects, the amount of decrease in the group given relatively minimal counseling was both surprising and gratifying in view of the previous difficulties with obtaining long-term behavioural changes to improve the cardiovascular risk status. These findings encourage adding counseling as an important early augmenting intervention to lifestyle modifications that may sustain beneficial therapeutic effect. This view is further supported with

the findings of the largest population-based experience of lifestyle modification as a strategy to reduce cardiovascular risk factors, CVD, and mortality. The study used a comprehensive community-level approach that encompassed the health and other services like voluntary organizations, local media, businesses including the Food Industry and changes to public policy. It demonstrated a reduction in mortality from coronary artery disease by 55% in men and by 68% in women over a 20-year period^[170]. Furthermore, in a recent randomized clinical trial^[171] it was found that subjects with increased BP who participated in an automated online self-management program resulted in improved BP among prehypertensive or hypertensive subjects. These findings emphasize the need to involve patients for a more sustainable outcome. Similar results were obtained in overt hypertensive patients, who, in a prospective cohort study received repeated nonpharmacological recommendations to follow low-salt and low-calorie diets and to do physical activities^[172]. This study concluded that adherence to follow low-salt and low-calorie diets is associated with clinically relevant long-term BP reduction and better hypertension control in clinical setting.

Although the evidence on reducing alcohol intake and reduction in BP is equivocal^[173,174], a meta-analysis of trials in this respect with many of the analysed trials included prehypertensives^[175] suggest that reducing alcohol intake can independently lower SBP.

THERAPEUTIC INTERVENTION WITH A MONODRUG THERAPY

All concluded studies that have been attempting to treat PHTN used one drug that affects the RAS in the form of ACEIs, ARBs or renin inhibitors. The use of other monotherapies such as β -blockers, was not shown to be, compared to ACEIs, effective in improving resistance arteries function after two years of use in human^[123,124]. The involvement of RAS in PHTN and HTN was discussed earlier. This part of the review summarizes the outcome of clinical trials using drugs that affect RAS in this respect.

Clinical trials with drugs affecting RAS: ongoing clinical trials

Two clinical trials are ongoing: (1) the first trial is the PREVER-Prevention trial^[176], a controlled randomized, double-blind trial designed to include individuals with PHTN given chlorthalidone 12.5 mg plus amiloride 2.5 mg or placebo. The study is to investigate if early use of drugs in individuals with PHTN may prevent cardiovascular events, target-organ damage and the incidence of overt HTN. In the 2nd International Conference on Prehypertension and Cardiometabolic Syndrome (January 31 - February 3, 2013; Barcelona, Spain) the trial co-principal investigator (Fuchs FD) announced that PREVER has finished enrollment of 1053 patients. According to the study design, patients who are still prehypertensive after three months of recommended lifestyle changes are randomized to a low-dose combination of chlorthalidone

plus amiloride or to placebo. Preliminary results from the study^[177] indicate that 659 (77%) of subjects remained prehypertensive and were randomized according to the study protocol; another 7.5% had abnormal lab values, and 6.2% had progressed to developing HTN, while 9% had seen their BP drop to within normal values; and (2) The second trial is the Chinese High Normal Blood Pressure (CHINOM) trial. The study has finished enrollment of 10689 patients with BP in the range of 130-139/85-89 mmHg and at least one other cardiovascular disease risk factor (but no established diabetes, renal or hepatic dysfunction, or history of stroke or CVD). The trial randomized patients to one of three parallel treatment groups: telmisartan 40 mg, indapamide 1.5 mg, or, in the third group, placebo or a combination pill of hydrochlorothiazide 12.5 mg, triamterene 12.5 mg, dihydralazine 12.5 mg, and reserpine 0.1 mg. The primary end point of the study is combined CV events (nonfatal stroke, nonfatal MI, and CVD death), while secondary end point addresses new-onset hypertension and new-onset diabetes. In the above mentioned conference, it was announced that the CHINOM trial is still awaiting the first results which may be still several years away. However, baseline characteristics of study subjects are showing that 70% of subjects enrolled actually have more than one cardiovascular risk factor with metabolic syndrome being the most common. More than three-quarters of participants are overweight or obese, 42% have high triglycerides, and over one-third have a family history of hypertension^[177].

Clinical trials with drugs affecting RAS: concluded clinical trials

The first clinical trial was the TRial Of Preventing Hypertension (TROPHY)^[178,179] which examined whether early treatment of PHTN justified pharmacologic intervention with the use of an ARB (candesartan 16 mg daily) in HTN. TROPHY hypothesis to examine whether ARBs may be useful to treat PHTN was based on the following: (1) PHTN is a strong independent predictor of cardiovascular events; (2) growth factors mediated by stimulation of the sympathetic nervous system^[180] and excess activity of RAS^[181] tend to promote vascular hypertrophy by direct as well as hemodynamic effects. Antihypertension treatment with ACEIs or ARBs, but not with β -blockers, has been reported to cause regression of arteriolar hypertrophy^[123,124]; and (3) despite intensive community efforts to promote healthy lifestyle, the prevalence of PHTN^[182] in the United States is increasing.

Over a period of four years of TROPHY study, it was found that stage 1 HTN developed in nearly two thirds of patients with untreated PHTN (the placebo group). Treatment of PHTN with candesartan appeared to be well tolerated and reduced the risk of incident HTN during the study period. The authors concluded that, treatment of PHTN appears to be feasible.

Although the observations in this study indicate that candesartan may ameliorate BP in prehypertensives, a comment by the authors stated that they do not advocate

treatment of the 25 million people (in the United States) with prehypertension. They added that additional studies will be needed to ascertain whether this or other strategies involving early pharmacologic treatment of PHTN would positively affect clinical outcomes.

Another trial is the PHARAO study^[183] which demonstrated that ramipril (an ACEI) given to prehypertensives reduced the risk of HTN by 34.4% compared to those not taking antihypertensive drugs; however, no differences were found in cardiovascular or cerebrovascular events. The study concluded that prehypertensives are more likely to progress to overt HTN than those with optimal or normal BP when treated with ACEIs.

A third trial, on the other hand, concluded that pharmacological therapy is indicated for some patients with PHTN who have specific comorbidities, including diabetes mellitus, chronic kidney disease and coronary artery disease^[184], while another trial^[185] did not support the use of antihypertensive drugs in “normotensive” subjects and that, ARBs might offer less protection against myocardial infarction than ACEs.

Most recently, the AQUARIUS trial^[186] examined the effect of aliskiren (a renin inhibitor) on progression of coronary atherosclerosis in a double-blind, randomized, multicenter trial study. It concluded that among participants with PHTN and coronary artery disease, the use of aliskiren compared with placebo did not result in improvement or slowing of progression of coronary atherosclerosis and that their findings do not support the use of aliskiren for regression or prevention of progression of coronary atherosclerosis.

INTERVENTION WITH MULTIDRUG FORMULATIONS: SHOULD THE “POLYPILL” BE CONSIDERED IN PHTN?

What is the “Polypill”?

The “Polypill” is a multidrug formulation with modified drug combinations containing drugs such aspirin, statins, β -blockers, ACEIs and ARBs; all of which are of proven value in reducing CVD morbidity and mortality. Approximately, half of the decline in cardiovascular mortality observed in developed countries during the last two decades is attributable to medical therapy using these types of drugs^[187].

The introduction of the Polypill idea was not intended for use in PHTN, rather it was for reducing the burden of CVD in economically disadvantaged individuals to reduce cost and improve adherence. It was meant to be applied to entire or large segments of the population. The reasons behind innovating the Polypill (see below) were, in effect, the same as those for intervening with PHTN, the authors suggest considering to include prehypertensives in future Polypill clinical trials to ascertain the potential benefit of using the Polypill in these subjects.

Rationale behind the polypill composition

Some of the main relevant reasons for introducing the

Polypill are summarized as follows: (1) cardiovascular disease is the major cause of death and disability globally and affects approximately half of all individuals over their lifetimes^[140]. CVD has increased in developing countries, and by the year 2020, 80% of the global CVD mortality is predicted to occur in low- and middle-income countries^[188]; (2) world population is threatened by increasing obesity, sedentary lifestyles, and diabetes mellitus rates^[187]. If these conditions are added to increased BP, primary intervention strategies directed towards community rather than individuals become of more therapeutic value; (3) nine to ten potentially modifiable risk factors account for 90% of the attributable risk for myocardial infarction and stroke, with similar estimates in all major regions of the world^[189,190]; and (4) the prevalence of low risk factor burden is on the increase. In the US it was only 4.4% during 1971-1975, 10.5% during 1988-1994, and 7.5% during 1999-2004^[191].

In addition to the above reasons, it has been shown that monodrug therapeutic intervention in PHTN has yielded mixed results, with some researchers have shown benefits^[184] while others have not^[185]. It seems that the existence of comorbidities determines how a prehypertensive subject is likely to respond to pharmacological intervention^[192]. Hence, the authors propose to consider including prehypertensives in future clinical trial of the Polypill to investigate how much benefit they may gain by multidrug therapeutic intervention.

Clinical evidence for the polypill effectiveness

Two randomized, placebo-controlled trials investigated the therapeutic effect of the polypill. The first was conducted in 2011^[193]. It was an international randomised placebo-controlled trial of a four-component combination pill ("polypill") in people with raised cardiovascular risk (over 7.5%, determined by the Framingham risk) using data on age, gender, BP, total cholesterol, HDL cholesterol, diabetes status, and cigarette smoking status. It contained aspirin 75 mg, lisinopril 10 mg, hydrochlorothiazide 12.5 mg, and simvastatin 20 mg or to placebo. The drug combination was associated with a 9.9-mmHg drop in SBP and a 0.8-mmol/L reduction in LDL cholesterol over a 12-wk treatment period.

The second clinical trial^[194] studied a polypill contained amlodipine 2.5 mg, losartan 25 mg, hydrochlorothiazide 12.5 mg and simvastatin 40 mg; but contained no aspirin. The pill was given for 12 wk. The treatment showed reductions mean systolic (17.9 mmHg) and DBP (9.8 mmHg) and LDL blood level was reduced by 1.4 mmol/L.

CONCLUSION

PHTN is a major health challenge that requires extra-attention. The "challenge" resides in finding the answer to "how/what" should be the intervention strategy (or strategies) that may best reduce its health impact.

In the search for a "strategy" to intervene in prehypertension, a number of considerations may be noteworthy

and can be summarized as follows: (1) the rationale behind therapeutic intervention in hypertensive, and, indeed, prehypertensive subjects is to prevent (or delay progression of) cardiovascular events and mortality caused by these conditions. Yet, it is equally accepted that presence of other comorbidities such as diabetes mellitus, obesity, dyslipidemia, *etc.* in addition to ethnic, age and gender differences should also be accounted for when an intervention strategy considered; (2) the term PHTN is based on "defining" HTN itself, which is established on a 20-mmHg per brackets. Yet, BP is confounded by many factors such as circadian rhythms, food intake, stress, exercise, emotional state *etc.* leading to "variable BP variability"^[137,195]; (3) based on the above two points, it is plausible to suggest that, categorizing and staging of subjects on basis of BP alone may need to be re-considered. It may be more clinically useful to contain factors, together, that cause BP variability to "stage" BP levels as well as to calculate cardiovascular risk factors. Such, may produce new terminologies or new definitions of PHTN and HTN. Consequently, an intervention strategy may not be "one-size-fits-all", and may necessitate more than one intervention. Different strategies (or combination thereof) may be considered. For example, males may require a different strategy from females since differences between genders have been reported in overt HTN^[196,197]. Similarly, children PB progression differs from that of adults^[198] and, thus may need a different intervention strategy. Furthermore, different ethnicities have shown different patterns in both progression of their BP as well as response to therapy and, hence, they may need different intervention strategies^[199-202]; (4) the first-line treatment for prehypertensives should be based on adoption of a healthy lifestyle, especially if there are other associated risk factors such as obesity, dyslipidemia, pre-diabetes or diabetes, excessive alcohol intake, sedentary lifestyle and smoking^[203] as well as salt-intake restriction^[152]. It is desirable if TLCs would be adopted by government and NGOs and may be "enforced" as a "policy" that is directed (in a way similar to the antismoking campaign) towards changing community behavior; and (5) if pharmacologic means will be used, such should not be confined to drugs affecting RAS, other drugs may be investigated to ascertain whether it is the mere reduction in BP that is benefitting prehypertensives or other effects.

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Peroxisome proliferator-activated receptors for hypertension

Daisuke Usuda, Tsugiyasu Kanda

Daisuke Usuda, Tsugiyasu Kanda, Department of Community Medicine, Kanazawa Medical University Himi Municipal Hospital, Himi-shi 935-8531, Toyama-ken, Japan

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Correspondence to: Daisuke Usuda, MD, MTM, Department of Community Medicine, Kanazawa Medical University Himi Municipal Hospital, 1130 Kurakawa, Himi-shi 935-8531, Toyama-ken, Japan. united19771108@yahoo.co.jp

Telephone: +81-766-741900 Fax: +81-766-741901

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Abstract

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily, which is composed of four members encoded by distinct genes (α , β , γ , and δ). The genes undergo transactivation or transrepression under specific mechanisms that lead to the induction or repression of target gene expression. As is the case with other nuclear receptors, all four PPAR isoforms contain five or six structural regions in four functional domains; namely, A/B, C, D, and E/F. PPARs have many functions, particularly functions involving control of vascular tone, inflammation, and energy homeostasis, and are, therefore, important targets for hypertension, obesity, obesity-induced inflammation, and metabolic syndrome in general. Hence, PPARs also represent drug targets, and PPAR α and PPAR γ agonists are used clinically in the treatment of dyslipidemia and type 2 diabetes mellitus, respectively. Because of their pleiotropic effects, they have been identified as active in a number of diseases and are targets for the development of a broad range of therapies for a variety of diseases. It is likely that the range of PPAR γ agonist therapeutic actions will result in novel approaches to lifestyle and other diseases. The combination of PPARs with reagents or with other cardiovascular drugs, such as diuretics and angiotensin II receptor blockers, should be studied.

This article provides a review of PPAR isoform characteristics, a discussion of progress in our understanding of the biological actions of PPARs, and a summary of PPAR agonist development for patient management. We also include a summary of the experimental and clinical evidence obtained from animal studies and clinical trials conducted to evaluate the usefulness and effectiveness of PPAR agonists in the treatment of lifestyle-related diseases.

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Key words: Peroxisome proliferator-activated receptors; Nuclear receptor; Isoform; mRNA; Blood pressure; Hypertension; Obesity; Angiotensin II receptor blocker; Diabetes mellitus

Core tip: Lifestyle-related diseases are major public health problem worldwide, and the prevalence of these diseases and subsequent complications has increased rapidly over the past 20 years. It has been a decade or more since the first report of the pleiotropic effects of peroxisome proliferator-activated receptors (PPARs), and numerous studies on their novel effects continue to appear every month. In addition to their effects on blood pressure, atherosclerosis, and kidney dysfunction, anti-cancer effects of PPAR γ ligands have been reported recently. The effectiveness of PPAR agonists in the treatment of lifestyle-related diseases will be increasingly appreciated. This review summarizes the current literature on PPARs.

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INTRODUCTION

PPARs are ligand-activated transcription factors of the

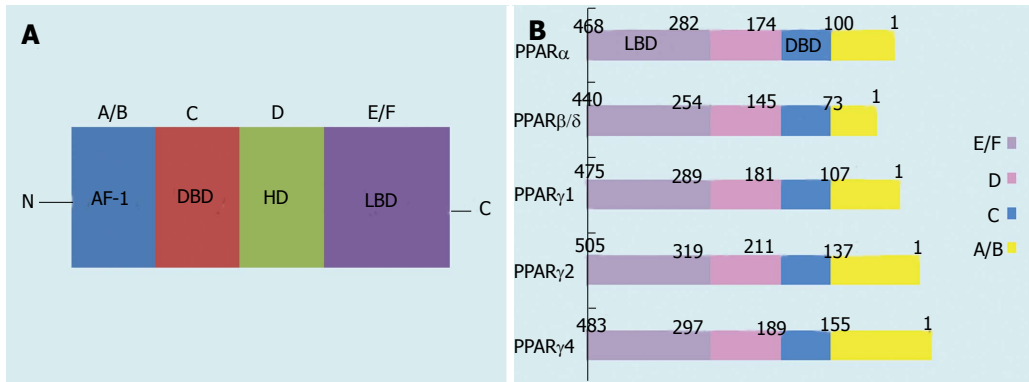


Figure 1 Schematic structure of peroxisome proliferator-activated receptor protein isoforms. A/B, C, D, and E/F indicate the N-terminal A/B domain containing a ligand-independent AF-1, the DBD, the hinge region, and the C-terminal LBD containing AF-2, respectively. AF-1 is responsible for phosphorylation, while AF-2 promotes the recruitment of co-activators for gene transcription. PPAR: Peroxisome proliferator-activated receptor; AF-1: Activation function-1; DBD: DNA-binding domain; HD: Hinge domain; LBD: Ligand-binding domain. Figure adapted from reference^[6].

nuclear receptor superfamily, and they comprise four members encoded by distinct genes (α , β , γ and δ). The PPARs undergo transactivation or transrepression under distinct mechanisms that lead to the induction or repression of target gene expression^[1]. PPARs bind to sequence-specific target elements in the promoter region of target genes following heterodimerization with the retinoid receptor, and in doing so, they control the majority of steps in cellular fatty acid uptake, utilization, oxidation, and storage pathways; cell growth and migration; oxidative stress; and inflammation in the cardiovascular system^[1,2]. Each PPAR is primarily located in a distinct set of tissues, is stimulated by different ligands, and has different effects^[2]. Certain new effects of PPARs on hypertension have been identified in recent studies, and the present mini-review focuses on the literature related to the effects that PPARs and their agonists exert in this area. Each member of the PPAR family possesses distinct functions that are determined by their ligand affinity, expression, and activity, which are dependent on the metabolic pathway and the type of tissue.

PPAR STRUCTURE

PPARs are orphan nuclear receptors belonging to the steroid, retinoid, and thyroid hormone receptor superfamily of ligand-activated transcription factors^[3,4]. Three distinct receptor types have been cloned and characterized: PPAR α (NR1C1), PPAR β/δ (NR1C2), and PPAR γ (NR1C3)^[5]. Like other nuclear receptors, these PPAR isoforms have five or six structural regions within four functional domains, termed A/B, C, D, and E/F (Figure 1A)^[5]. The variable NH₂-terminal end, which is a ligand-independent transactivation domain (the A/B domain), contains activation function (AF)-1, which is a target of kinase phosphorylation^[5]. The 70-amino-acid-long PPAR DNA-binding domain (the C domain) contains two highly conserved zinc finger motifs and promotes the binding of receptors to a DNA sequence in the promoter region of target genes, which is known as the peroxisome proliferator response element (PPRE)^[5]. The hinge region

(the D domain) acts as a docking site for cofactors. The C-terminal or ligand-binding domain (the E/F domain) is responsible for ligand specificity and the activation of PPAR binding to the PPRE, which increases target gene expression. The E/F domain uses cofactors for the transactivation *via* the ligand-dependent trans-AF-2^[5]. When activated by endogenous or synthetic ligands, the PPARs, like other nuclear hormone receptors, heterodimerize with the 9-*cis*-retinoic acid receptor (retinoid \times receptor)^[5]. The PPAR-retinoid \times receptor heterodimer undergoes conformational changes, binds to the PPRE in the promoter region of the target gene, and alters coactivator/corepressor dynamics to modulate the transcription machinery, which in turn affects the initiation of transcription (*via* upregulation or downregulation) and the abundance of messenger RNA (mRNA) in the target genes^[6,7]. PPARs are also drug targets; currently, PPAR α agonists (fibrates) are in clinical use for treating dyslipidemia, and PPAR γ agonists (thiazolidinediones (TZDs)) are being used to treat type 2 diabetes mellitus (T2DM)^[8].

PPAR EXPRESSION

The PPAR family possesses distinct functions that are determined by their ligand affinity, expression, and activity, which are dependent on the metabolic pathway and the type of tissue^[1]. The characteristics of each PPAR isotype are described below.

PPAR α was the first PPAR isotype to be cloned, and its name comes from its activation by peroxisome proliferator chemicals^[9,10]. Its expression is greatest in tissues with a high fatty acid oxidation rates, such as heart, liver and skeletal muscle, and functions as a major regulator of fatty acid homeostasis^[10-13]. PPAR α expression is also significant in the adipose, adrenal and kidney tissue (particularly brown adipose tissue), and the majority of cell types, including endothelial, smooth muscle, and macrophages, in the vasculature^[12-14].

PPAR β/δ (PPAR δ) is expressed at relatively high levels in liver, kidney, cardiac and skeletal muscle, adipose tissue, brain, colon, and vasculature^[14-17]. Unlike PPAR α



Figure 2 Domain structure of the peroxisome proliferator-activated receptor γ isoforms. PPAR: Peroxisome proliferator-activated receptor. Figure adapted from reference^[8].

and PPAR γ , PPAR δ does not seem to be the target of available drugs^[8]. The unavailability of PPAR δ -targeted drugs may be due to its wide ranging expression. The physiological function of PPAR δ is much less studied and understood^[8].

PPAR γ is highly expressed in adipose tissue and plays an indispensable role in the regulation of adipocyte differentiation, lipid storage, and glucose metabolism and in the transcriptional regulation of a number of genes involved in these metabolic processes^[13,18-20]. Some key target genes of PPAR γ include the fat-specific *ap2* gene, LPL, fatty acid transport, fatty acid-binding protein, FAT/CD36, acyl-CoA synthase, GLUT4, glucokinase, phosphoenolpyruvate carboxykinase, uncoupling proteins 1, 2, and 3 and LXR α ^[18,19,21]. PPAR γ also regulates genes involved in insulin signaling and the expression of proinflammatory cytokines such as tumor necrosis factor (TNF)- α ^[20,21]. It also has significant anti-inflammatory effects^[18,19,21] <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3246744/> - R49. Most importantly, PPAR γ is a well-recognized cellular target for the antidiabetic thiazolidinediones, which sensitize cells to insulin and improve insulin sensitivity and action^[22-24]. To date, seven mRNA transcripts generated through different forms of initiation and the alternative splicing of five exons at the 5'-terminal region (A1, A2, B, C and D) have been identified (Figure 2)^[24-26]. Each mRNA transcript is different, based on the combination of five exons. They have been designated PPAR γ 1, - γ 2, - γ 3, - γ 4, - γ 5, - γ 6, and - γ 7. PPAR γ 1, - γ 3, - γ 5, and - γ 7 mRNA transcripts translate to the identical PPAR γ 1 protein. PPAR γ 2 mRNA yields PPAR γ 2 protein, while PPAR γ 4 and - γ 6 mRNA transcripts produce identical PPAR γ 4 protein (Figure 1B)^[25-27]. The PPAR γ 1 mRNA isoform is expressed in a range of tissues: cardiac and skeletal muscle; pancreatic β -cells; the spleen, intestines, kidneys, and adrenal gland; vascular cells such as endothelial cells (ECs) and smooth muscle cells; and monocytes/macrophages^[26,28,29]. The expression of PPAR γ 2 mRNA is primarily restricted to adipose tissue, whereas PPAR γ 3 mRNA is abundant in macrophages, the large intestine (colon), and adipocytes^[24,26,30]. High levels of PPAR γ 4, - γ 5, - γ 6, and - γ 7 mRNA tran-

scripts are expressed in macrophages, while PPAR γ 6 and - γ 7 mRNAs are also detected in adipose tissue^[24,25,27,30].

PPAR ACTIVATION AND REGULATORY ROLES

PPARs are found primarily within the nucleus without their ligand and localize to target gene promoters with either co-activator or co-repressor complexes^[31]. To date, many ligands had been identified that activate and modulate PPAR functions^[31]. Endogenous lipid metabolites from saturated or unsaturated fatty acids, for example, are able to bind to nuclear receptors and activate or repress gene expression^[31]. Another group of PPAR ligands consists of lipid metabolites from essential fatty acids, such as arachidonic acid derived from lipoxygenase or cyclooxygenase activity^[31]. In particular, the best-characterized endogenous ligands known to stimulate PPAR α are the eicosanoids LTB4 and 8-hydroxyeicosatetraenoic acid (8(S)-HETE), while 15d-PGJ2 and 13-HODE activate PPAR γ ^[31]. Other essential fatty acid metabolites, such as 15-HETE, have been suggested to activate PPAR β / δ ^[31].

The discovery of PPARs as a key regulator of metabolic pathways has provided significant insight into the mechanisms involved in this process^[28,32]. PPARs act as nutritional sensors that regulate a variety of homeostatic functions, including metabolism, inflammation, and development^[28,32,33]. PPARs are involved in many functions, particularly those having to do with the regulation of vascular tone, inflammation, and energy homeostasis. Therefore, they represent important targets for addressing hypertension, obesity, obesity-induced inflammation, and metabolic syndrome in general^[1,32-34]. PPARs may influence the inflammatory response either directly through the transcriptional downregulation of proinflammatory genes *via* mechanisms involving transrepression or indirectly *via* their transcriptional effects on lipid metabolism^[1,8]. Because of their pleiotropic effects, they are now known to be active in a number of disease conditions, and they represent potent therapeutic targets for a wide range of diseases^[1,8,32,34]. PPAR agonists may be of benefit, either alone or in combination with other drugs that influence the inflammatory response, in treating hypertension, atherosclerosis, and metabolic derangements associated with obesity^[34].

The endogenous ligands that bind to PPAR α with the highest affinity are saturated/unsaturated fatty acids, leukotriene derivatives, and VLDL hydrolysis products^[33]. Examples of synthetic ligands that bind PPAR α are the fibrate class of hypolipidemic drugs, the experimental ligand Wy-14643 ([4-chloro-6-(2,3-xyldino)-2-pyrimidinylthio] acetic acid) and some phthalate monoesters (monoethylhexyl phthalate), and herbicides (lactofen)^[33]. PPAR α is a major regulator of the mitochondrial and peroxisomal β -oxidation pathway, and as will be discussed below, it is suggested that these pathways are involved in the pathogenesis of various liver complications^[33]. PPAR α activation inhibits vascular smooth muscle pro-

inflammatory responses, attenuating the development of atherosclerosis^[15,35]. PPAR α ligands negatively regulate interleukin (IL)-6 promoter activation, and chronic treatment with fenofibrate, a PPAR α agonist, suppresses IL-6-induced atherosclerosis^[36]. The absence of PPAR α expression is suggested to prolonged the inflammatory response, and PPAR α has anti-inflammatory properties^[36]. Furthermore, the PPAR α ligand, fenofibrate, may repress ICAM-1 and VCAM-1 expression in endothelial cells^[36]. In addition, PPAR α activation has been reported to inhibit NF- κ B activation and inflammatory gene expression^[35].

Activation of the nuclear hormone receptor PPAR β / δ is known to both improve insulin resistance and plasma high-density lipoprotein levels and to exhibit anti-inflammatory properties in the vessel wall through the inhibition of vascular cell adhesion molecule 1 and monocyte chemoattractant protein 1 expression^[37].

Although PPAR γ was first to be recognized as an anti-inflammatory agent, both PPAR α and PPAR δ are also known to have similar effects^[34]. Inflammation is a significant aspect of the damage that hypertensive disease causes^[34]. PPARs are now seen as important determinants of macrophage polarization^[34]. Monocyte precursors of classically and alternatively activated macrophages are being identified as important participants in the progress of metabolic syndrome-related cardiovascular disease, including hypertension, hyperlipidemia, and obesity^[8,38,39]. The activation of PPAR β / δ has been shown to increase lipid catabolism in the skeletal muscle, heart, and adipose tissue and to improve the serum lipid profile and insulin sensitivity^[39,40]. Further, PPAR β / δ ligands stop weight gain and reduce macrophage-derived inflammation^[40]. One new approach that may prevent or regress hypertension-induced vascular, renal, and, perhaps, brain changes is the activation of nuclear receptors, which not only have metabolic effects but also exert anti-inflammatory actions through PPAR α and PPAR γ ^[41]. PPAR α and PPAR γ are therapeutic targets for hypertriglyceridemia and insulin resistance, respectively^[42,43].

Covalent modifications include phosphorylation, ubiquitylation, O-GlcNAcylation, and SUMOylation^[44]. Covalent modifications of PPAR γ are key regulatory mechanisms that control both PPAR γ protein stability and transcriptional activity^[39,44]. PPAR γ functions as a master switch in controlling adipocyte differentiation and development, and its activation has an important role in glucose metabolism by enhancing insulin sensitization^[39,45]. PPAR γ is a primary target for TZD-structured insulin sensitizers such as pioglitazone and rosiglitazone, which are used in the treatment of T2DM^[39,45]. Additionally, PPAR γ activation inhibits adhesion cascades and detrimental vascular inflammatory events^[39,45]. Furthermore, although the primary action of select ARBs, which partially activate PPAR γ , is to lower blood pressure, they may also be effective in treating insulin resistance and dyslipidemia absent the toxicity associated with full PPAR γ agonists^[39,46].

PPAR γ activation is known to have an influence on

the events connected with the development and progression of atherosclerotic lesions^[14,15,24,34,39,47]. PPAR γ and its ligands may exert direct antiatherosclerotic action^[14,15,24,48-50]. Consistent with the anti-inflammatory properties of PPAR γ and the TZDs, aortas showed decreased accumulation of macrophages in the lesions as well as attenuated expression of proatherogenic agents. Interestingly, these changes occurred independently of improvements in dyslipidemia, glycemic control, and hypertension, which supports the assumption of a direct vascular effect^[8,50,51]. Moreover, PPAR γ activation plays a distinct role in regulating the physiology and expression of endothelial nitric oxide synthase (eNOS) in the endothelium, resulting in enhanced generation of vascular nitric oxide^[45]. PPAR γ activation-mediated vascular anti-inflammatory and direct endothelial functional regulatory actions could therefore be beneficial in improving vascular function in patients with atherosclerosis and hypertension with or without DM^[45]. Unfortunately, PPAR γ agonists can exert long-term effects on certain patients, including increased body weight, fluid retention, and risk of heart failure^[39]. This is unfortunate, as TZDs show consistent efficacy in the treatment of T2DM^[39]. More recently, there has been increased concern about the association between TZD and bone loss^[39]. The association with bone loss is an especially worrisome concern because fracture is usually when it is detected^[39]. The biguanide metformin is currently the first-line medication in the treatment of T2DM due to increasing concerns about the safety of TZDs^[39]. While the cardiac side effect profile of rosiglitazone-like PPAR γ full agonists is unfortunate, the therapeutic potential of novel pharmacological agents targeting PPAR γ submaximal cannot be excluded. Interestingly, newly synthesized partial agonists of PPAR γ , such as balaglitazone, MBX-102, MK-0533, PAR-1622, PAM-1616, KR-62776, and SPPAR γ M5, have a reduced tendency to cause the adverse effects associated with full agonists of PPAR γ or may be entirely devoid of such effects^[45]. Therefore, with as much as 50% of patients with ischemic stroke and transient ischemic attack also having insulin resistance, drugs capable of addressing both hypertension and insulin resistance could be of great benefit in preventing stroke^[46]. In summary, PPAR γ is implicated both in the maintenance of vascular homeostasis and in the pathogenesis of a number of vascular conditions such as atherosclerosis, hypertension, and restenosis^[28,29,39,52]. TZDs, which are PPAR γ agonists, lower blood pressure and exert protective vascular effects through largely unknown mechanisms^[39,52]. In contrast, loss-of-function dominant-negative mutations in human PPAR γ cause insulin resistance and severe early onset hypertension^[52].

EFFECTS OF PPARS ON BLOOD PRESSURE

PPAR α ligands have been reported to decrease blood pressure in various models of hypertension^[36]. Several

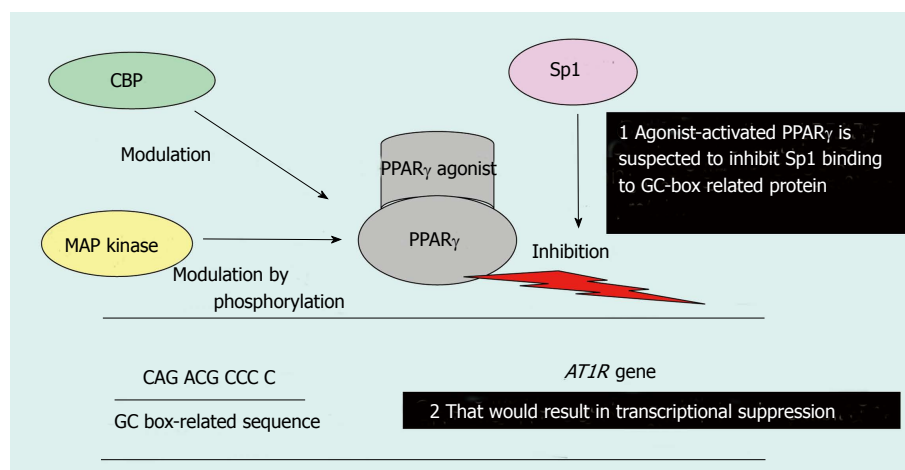


Figure 3 Possible mechanism of peroxisome proliferator-activated receptor γ -agonist-mediated transcriptional suppression of the Ang-II type 1 receptor gene promoter. PPAR: Peroxisome proliferator-activated receptor; AT1R: Ang-II type 1 receptor; CBP: CERE-1 binding protein; MAP: Mitogen-activated protein. Figure adapted from reference^[71].

mechanisms have been proposed for the antihypertensive effects of PPAR α agonists such as the increased excretion of Na⁺ through reduced Na⁺-K⁺ ATPase activity in the proximal tubules, increased cytochrome P450 (CYP) 4A expression, and increased renal tubular 20-HETE production, which exerts a natriuretic effect^[36,53-57]. A recent report has described a crosstalk between PPAR α and IL-6 in the regulation of blood pressure^[36,58]. Furthermore, another report demonstrates that PPAR α activation attenuates angiotensin-II (Ang-II)-induced hypertension through the upregulation of CYP4A and CYP2J and the attenuation of plasma IL-6, renal MCP-1 and other inflammatory markers, and the renal expression of ICAM-1 and COX-2^[36].

A PPAR β/δ agonist has been reported to induce progressive systolic arterial blood pressure and heart rate reduction, and to reduce mesenteric arterial remodeling, endothelial dysfunction, and aortic vasoconstriction in response to Ang-II^[37]. These were accompanied by a significant increase in eNOS activity attributed to upregulated eNOS and downregulated caveolin 1 protein expression^[37]. Moreover, the PPAR β/δ agonist also inhibited vascular superoxide production, downregulated p22^{phox} and p47^{phox} protein expression, decreased both basal and Ang-II-stimulated NADPH oxidase activity, inhibited extracellular-regulated kinase 1/2 activation, and reduced the expression of proinflammatory and proatherogenic genes, including IL-1 β , IL-6, and intercellular adhesion molecule 1^[37]. Further, the same study showed that PPAR β/δ activation, both *in vitro* and *in vivo*, increased the expression of RGS4 and RGS5, which are regulators of G protein-coupled signaling proteins; RGS4 and RGS5, in turn, negatively modulated the vascular actions of Ang-II^[37]. PPAR β/δ activation also exerted antihypertensive effects, restored vascular structure and function, and reduced the oxidative, proinflammatory, and proatherogenic statuses^[37]. Hence, PPAR β/δ was proposed as a new therapeutic target in hypertension^[37].

It has been reported recently that independent of its blood glucose-lowering effects, PPAR γ demonstrates pleiotropic beneficial effects on vasculature^[59]. The effect may possibly be due to PPAR γ -mediated inhibition of Ang-II type 1 receptor (AT1R) expression as well as

Ang-II-mediated signaling pathways, which may result in suppression of the renin-angiotensin system (RAS) and lead to a lower blood pressure^[59].

However, it has also been speculated that PPAR γ -induced AT1R gene transcription suppression is mediated through the inhibition of Sp1 binding to DNA. This inhibition is due to the protein-protein interaction between ligand-activated PPAR γ and Sp1; indeed, the PPAR γ ligand-mediated suppression of AT1R expression has been demonstrated previously (Figure 3)^[60-62]. Interestingly, transcription suppression was abrogated by the over-expression of the coactivator CERE-1 binding protein (CBP) and PPAR γ phosphorylation by mitogen-activated protein (MAP) kinase, most likely because of the functional modification of PPAR γ (Figure 3)^[60]. Moreover, PPAR γ ligands have been shown to suppress Ang-II-induced phosphatidylinositol 3-kinase and MAP kinase and to ameliorate Ang-II-mediated inflammatory responses by interfering with the Toll-like receptor 4-dependent signaling pathway^[62,63]. Therefore, PPAR γ not only downregulates AT1R expression but also inhibits Ang-II-mediated signaling pathways, which may result in RAS suppression (Figure 4)^[62-64]. On the other hand, transgenic mice expressing a dominant negative PPAR γ P465L mutation are hypertensive, which is consistent with the phenotype of patients who have an equivalent PPAR γ P467L mutation without affecting components of the RAS^[59]. Thus, ligand-activated PPAR γ may lower blood pressure through several different mechanisms in addition to inhibiting the RAS^[59].

In terms of blood pressure, the transient administration of ARBs may prevent the development of hypertension, and high doses of ARBs may regress mild hypertension^[65]. Next-generation ARBs are becoming available that are intended not only to antagonize AT1R but also to block endothelin receptors, function as nitric oxide donors, inhibit neprilysin activity, increase natriuretic peptide levels, or stimulate PPAR γ ^[66]. It has been shown that ARBs have benefits beyond their established cardioprotective and vasculoprotective effects, including lowering risk of new-onset diabetes and its associated cardiovascular effects^[67]. Furthermore, it has also been found that the drug telmisartan can selectively activate PPAR γ in

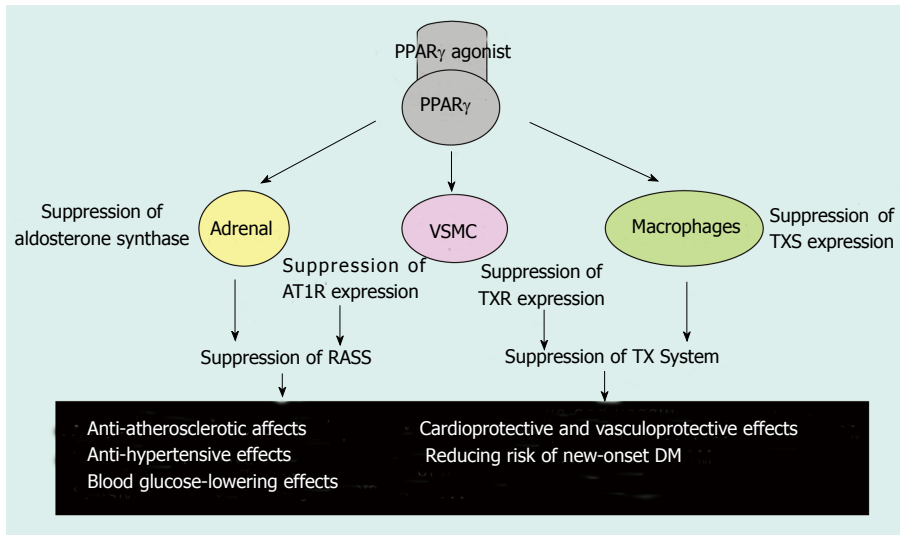


Figure 4 Possible effects of Peroxisome proliferator-activated receptor γ agonists. PPAR: Peroxisome proliferator-activated receptor; AT1R: Ang- II type 1 receptor; RAAS: Renin-angiotensin-aldosterone system; TX: Thromboxane; TXS: TX synthase; TXR: TX receptor; VSMC: Vascular smooth muscle cells; DM: Diabetes mellitus. Table adapted from reference^[71].

targeting DM, and it therefore provides an approach to the prevention and treatment of cardiovascular complications in high-risk elderly patients suffering from hypertension and new-onset DM^[67]. The beneficial metabolic effects of telmisartan have been attributed to its action as an Ang- II receptor blocker and as a partial PPAR γ agonist, and it has also been found that telmisartan may have the strongest binding affinity to AT1R^[43,68]. Treatment with telmisartan has been shown to significantly improve endothelial dysfunction and inhibit lipid accumulation in the liver^[43]. It is possible that the favorable characteristics of telmisartan are due to its action as a partial PPAR γ agonist, apart from its blood pressure-lowering effect as an Ang- II blocker, possibly earning it the name “metabosartan”^[43]. These observations suggest that because of its unique PPAR γ -modulating activity, telmisartan may be one of the most promising sartans for the treatment of cardiometabolic disorders^[68].

EVIDENCE FROM ANIMAL AND HUMAN STUDIES

PPAR γ activation is suggested to be beneficial in inflammatory diseases, not only in humans but also in rats and pigs^[69]. The question is now whether PPAR γ activation mitigates immunological stress such as mastitis in livestock. In livestock species in general, however, data on the use of synthetic PPAR agonists are limited^[69]. Considering the high amino acid identities ranging from 95% to 98% for the PPAR proteins in all species, one may believe that bovine and porcine PPARs could also be targeted using the existing synthetic PPAR agonists^[69]. However, because only a minor overlap between the Wy-regulated genes from mouse and human primary hepatocytes was found and because PPAREs are not fundamentally conserved among species, activation of the PPARs does not necessarily activate the same array of genes in one species as in another^[69]. Data from the literature makes it clear that further studies on the impact of PPAR ligands in livestock are necessary as such investigations may identify

unconsidered health and sanitation benefits.

It has been shown that WY14643, a potent PPAR α agonist, has cardioprotective and cardiodepressive effects when used to treat encephalomyocarditis virus-induced myocarditis in diabetic mice^[38]. The cardioprotective effect may be due to its anti-inflammatory properties and its ability to increase cardiac adiponectin expression, whereas the reduced cardiac efficiency may be due to its enhancement of cardiac UCP3 mRNA expression^[38]. In animals, the pharmacological or genetic elevation of plasma adiponectin relieves obesity-induced endothelial dysfunction and hypertension, and prevents atherosclerosis, myocardial infarction, and diabetic cardiomyopathy^[70]. These therapeutic benefits of PPAR γ agonists (TZDs) are mediated by the induction of adiponectin^[70]. Adiponectin protects cardiovascular health through its vasodilator, anti-apoptotic, anti-inflammatory, and anti-oxidative activities in both cardiac and vascular cells^[70].

PPAR γ agonists are known to lower blood pressure in humans, possibly through the suppression of the RAS, by mechanisms including the inhibition of AT1R expression, Ang- II-mediated signaling pathways, and Ang- II-induced adrenal aldosterone synthesis/secretion^[52,71]. PPAR γ agonists also inhibit the progression of atherosclerosis in humans, possibly through a pathway involving suppression of the RAS and the thromboxane system, as well as the protection of endothelial function^[71]. Moreover, PPAR γ -agonist-mediated renal protection, particularly the reduction of albuminuria, has been reported in diabetic nephropathy, including animal models of the disease, and in nondiabetic renal dysfunction^[71]. The renal protective activities may reflect, at least in part, the ability of PPAR γ agonists to lower blood pressure, protect endothelial function, and cause vasodilation of the glomerular efferent arterioles^[71]. In addition, it has recently been reported that PPAR γ agonists have antineoplastic effects and that they can ameliorate polycystic kidney, polycystic liver, and cardiac defects through the β -catenin, c-Myc, CFTR, MCP-1, S6, ERK, and TGF- β signaling pathways in animal models of chronic kidney disease (CKD)^[71]. The multiple therapeutic actions of PPAR γ agonists

leave no doubt that they will produce new approaches to lifestyle-related and other diseases^[71].

However, negative (harmful) aspects of PPARs have also been reported. TZDs are insulin-sensitizing anti-diabetes agents that act through PPAR γ to cause a durable improvement in glycemic control in patients with T2DM^[72,73]. These benefits must be weighed against the side effects of the drug, which include weight gain, fluid retention, atypical fractures, and possibly, bladder cancer^[72,73]. Despite having similar effects on glycemic control, pioglitazone and rosiglitazone appear to have different effects on cardiovascular outcomes^[72,73]. Rosiglitazone has been associated with an increased risk of myocardial infarction, and its use in the United States is restricted because of cardiovascular safety concerns^[72,73]. PPAR- α/γ or - γ/δ dual agonists are now under development^[74,75].

As the literature has been indicating, disorders of pregnancy, such as preeclampsia and gestational diabetes, are potential targets for treatment with PPAR ligands^[76]. In clinical cases, including preeclampsia, gestational diabetes, and intrauterine growth restriction, aberrant regulation of components of the PPAR system parallels the dysregulation of metabolism, inflammation, and angiogenesis^[76]. These actions are the result of the roles of the PPARs in regulating human trophoblast invasion and early placental development^[76]. PPARs are involved in trophoblast invasion, placental development, parturition, and pregnancy-specific diseases, particularly preeclampsia and gestational diabetes^[76]. The PPAR system's involvement in pregnancy under physiologic and pathologic conditions has yet to be fully clarified due to a lack of knowledge about endogenous PPAR ligands^[76]. Partially characterized inflammatory, angiogenic, and metabolic disturbances in pregnancy-related diseases suggest that these synthetic PPAR agonists may be of potential use in these conditions^[76].

To date, several large clinical trials of hypertension using PPAR agonists have been conducted worldwide, including in Japan. The Losartan Intervention for Endpoint reduction in hypertension (LIFE) study compares the effects of losartan- (a PPAR γ agonist) and atenolol- (a β blocker) based antihypertensive treatment on cardiovascular morbidity and mortality in a population of 9193 hypertensive patients with left ventricular hypertrophy (LVH)^[77]. In the LIFE study, losartan-based treatment further reduced the primary composite end point (cardiovascular death, myocardial infarction, or stroke) by 13% [relative risk reduction (RRR) 0.87, 95%CI: 0.77-0.98, $P = 0.021$]. The further reduction in stroke with losartan (RRR 0.75, 95%CI: 0.63-0.89, $P = 0.001$) was the major contributing factor to the reduction in the primary end point^[77].

The Study on Cognition and Prognosis in the Elderly (SCOPE) assessed the effect of candesartan (PPAR γ agonist) on cardiovascular and cognitive outcomes in elderly patients (aged 70-89 years) with mild to moderate hypertension^[78]. Patients were randomized to candesartan 8-16 mg daily ($n = 2477$) or placebo ($n = 2460$) and followed for an average of 3.7 years^[78]. Other antihypertensive drugs were

added if blood pressure remained greater than 160 mmHg systolic and/or 90 mmHg diastolic^[78]. Due to extensive add-on therapy, particularly in patients randomized to placebo, the between-treatment difference in blood pressure was only 3.2/1.6 mmHg^[78]. The main analysis showed, however, that non-fatal stroke was reduced by 28% ($P = 0.04$) in the candesartan group compared with the control group, and a non-significant 11% reduction in the primary endpoint of major cardiovascular events was seen ($P = 0.19$)^[78]. In conclusion, the findings of SCOPE suggest that candesartan treatment reduces cardiovascular morbidity and mortality in old and very old patients with mild to moderate hypertension. Candesartan-based antihypertensive treatment may also have positive effects on cognitive function and quality of life^[78].

The Valsartan (PPAR γ agonist) Antihypertensive Long-term Use Evaluation (VALUE) trial was designed to evaluate the hypothesis that for the same blood-pressure control, valsartan would reduce cardiac morbidity and mortality more than amlodipine (a calcium channel blocker) in hypertensive patients at high cardiovascular risk^[79]. Blood pressure was reduced by both treatments, but the effects of the amlodipine-based regimen were more pronounced, particularly in the early period (blood pressure 4.0/2.1 mmHg lower in the amlodipine group than the valsartan group after 1 mo; 1.5/1.3 mmHg after 1 year; $P < 0.001$ between groups)^[79]. There was no difference between the treatment groups in the primary composite endpoint, which was the occurrence of cardiac disease^[79].

The Trial of Preventing Hypertension (TROPHY) investigated whether pharmacological treatment of prehypertension prevents or postpones stage 1 hypertension^[80]. Participants with repeated blood pressure measurements of 130-139 and/or 85-89 mmHg were randomly assigned to 2 years of candesartan or placebo, followed by 2 years of placebo for all^[80]. The 4-year incidence of hypertension was significantly ($P < 0.01$) lower than that previously reported in the placebo (-11.3%) and candesartan (-11.0%) groups^[80]. During the first 2 years, hypertension developed in 162 placebo and 53 candesartan participants (RRR 68%, $P < 0.001$)^[80]. After 4 years, hypertension occurred in 197 placebo and 165 candesartan participants (RRR 18%, $P < 0.009$)^[80]. The new definition resulted in a lower incidence of hypertension, but the outcomes were remarkably similar with both definitions and confirmed our original findings^[80].

In the Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial and the Telmisartan Randomized Assessment Study in ACE-I Intolerant Subjects with Cardiovascular Disease, researchers assessed the cardioprotective and antidiabetic effects of telmisartan^[67]. The collective data suggest that telmisartan is a promising drug for controlling hypertension and reducing vascular risk in high-risk elderly patients with new-onset diabetes^[52]. Furthermore, several clinical studies have demonstrated the blood pressure-lowering effect of TZDs as PPAR γ ligands^[81]. The recent PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive Study), which included 5238 T2DM enroll-

ees, also demonstrated a significant decrease in systolic blood pressure (3 mmHg) following treatment with pioglitazone (a TZD)^[82].

Disappointingly, the results from the Fenofibrate Intervention and Event Lowering in Diabetes trial failed to show a reduction in risk for the primary end-point (coronary heart disease death and nonfatal myocardial infarction) of coronary events with fenofibrate therapy^[83]. There are many explanations for these results, including the use of a low cardiovascular risk diabetic population; however, more investigation is clearly needed to understand the clinical relevance of fibrates for treating CVD^[83].

In a sub-analysis of the Candesartan Antihypertensive Survival Evaluation in Japan trial, researchers examined the relationship between the achieved blood pressure and cardiovascular events in hypertensive patients with T2DM, CKD, or LVH at baseline^[84]. A higher achieved blood pressure was associated with an increased risk of cardiovascular events in hypertensive patients with complications (T2DM, CKD, or LVH)^[84]. In patients with LVH, who achieved a systolic/diastolic blood pressure (SBP/DBP) < 130/75-79 mmHg, the risk of cardiovascular events was reduced to the same level as in those without LVH, an SBP/DBP < 130/75-79 mmHg^[84]. However, the risks of cardiovascular events in patients with DM or CKD, who achieved an SBP/DBP < 130/75-79 mmHg, were still significantly higher than in those without DM or CKD^[84].

CONCLUSION

Although a decade or more has passed since the pleiotropic effects of PPAR γ were first reported, numerous studies on its novel effects continue to appear each month. In addition to the effects on blood pressure, atherosclerosis, and kidney dysfunction described above, anti-cancer effects of PPAR γ ligands have recently been reported^[59]. The usefulness and effectiveness of PPAR γ ligands in the treatment of lifestyle-related diseases will be increasingly appreciated^[59,85].

Further refinement of experimental strategies, group-specific chemical modification of potential compounds, and the development of specific and reliable translational models and biomarkers to better understand their safety and efficacy should all be of great assistance in the future clinical development of novel types of PPAR agonists^[8]. Moreover, future efforts to further delineate the physiology, pharmacology, and molecular functions of the PPARs may identify additional novel targets that can also be exploited in the development of superior, efficacious, and tissue-/PPAR isotype-specific agonists for the treatment of hypertension^[8].

There are clearly many uncertainties about the use of PPAR agonists in the treatment of cardiovascular disease. They have highly complex biologic effects resulting from the activation or suppression of dozens of genes, and the biologic effects of the protein targets for most of these genes remain largely unknown. Moreover, they possess

different properties for different species^[2]. Further efforts to completely investigate the effects of the PPARs and their agonists and the mechanisms by which they improve lifestyle-related diseases are required, including high blood pressure, in both human and animal models^[2]. Additionally, the adverse effects of PPAR γ agonists on cardiac function and water retention and the mechanisms responsible for these effects should be clarified in detail, particularly in humans^[2]. Finally, the combination of PPARs with reagents or with other cardiovascular drugs such as diuretics and ARBs should be studied^[2].

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WJC 6th Anniversary Special Issues (2): Coronary artery disease

Genetics of coronary heart disease with reference to *ApoAI-CIII-AIV* gene region

Suraksha Agrawal, Sarabjit Mastana

Suraksha Agrawal, Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow UP 226014, India

Sarabjit Mastana, Human Genomics Lab, School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough LE11 3TU, United Kingdom

Author contributions: Agrawal S and Mastana S wrote the initial draft and were involved in editing and collation of tables and figures.

Correspondence to: Sarabjit Mastana, PhD, Human Genomics Lab, School of Sport, Exercise and Health Sciences, Loughborough University, Ashby Road, Loughborough LE11 3TU, United Kingdom. s.s.mastana@lboro.ac.uk

Telephone: +44-1509-223041 Fax: +44-1509-226301

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ability of these genes. Genome wide associations show different chromosomal locations which dock, earlier unknown, genes which may attribute to CAD. In the present review different *ApoAI-CIII-AIV* gene clusters have been discussed.

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Key words: *ApoAI-CIII-AIV* gene cluster; Haplotype analysis; Single nucleotide polymorphism; Candidate gene study; Genome wide association studies

Core tip: Cardiovascular disease analysis requires holistic approach using genomic, epigenomic and exposomic techniques to improve the quality of life of patients and contribution towards personalised medicine.

Abstract

Cardiovascular diseases are affected by multiple factors like genetic as well as environmental hence they reveal factorial nature. The evidences that genetic factors are susceptible for developing cardiovascular diseases come from twin studies and familial aggregation. Different ethnic populations reveal differences in the prevalence coronary artery disease (CAD) pointing towards the genetic susceptibility. With progression in molecular techniques different developments have been made to comprehend the disease physiology. Molecular markers have also assisted to recognize genes that may provide evidences to evaluate the role of genetic factors in causation of susceptibility towards CAD. Numerous studies suggest the contribution of specific "candidate genes", which correlate with various roles/pathways that are involved in the coronary heart disease. Different studies have revealed that there are large numbers of genes which are involved towards the predisposition of CAD. However, these reports are not consistent. One of the reasons could be weak contribution of genetic suscep-

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INTRODUCTION

Coronary artery disease (CAD), is mostly fatal if remain untreated result into atherosclerosis in the epicardial coronary arteries^[1]. Atherosclerotic plaques progressively narrow the coronary artery lumen and impair antegrade myocardial blood flow. This reduction in coronary artery flow may lead to a myocardial infarction.

Cardiovascular disease is a multifarious disorder showing large diversity of phenotypes. The accurate, and analogous phenotypic evidences are crucial for detailed understanding of the affiliation between disease and genes, as well as understanding the role of various extrinsic factors on different component of various genotypes.

Table 1 Prevalence of coronary artery disease in different Indian surveys

City	Prevalence	Ref.
Urban population		
Chandigarh	(6.60%)	Sarvotham <i>et al</i> ^[49]
Rohtak	(3.80%)	Gupta <i>et al</i> ^[50]
Jaipur	(7.60%)	Gupta <i>et al</i> ^[51]
Delhi	(9.70%)	Chada <i>et al</i> ^[52]
Rural population		
Jaipur	(3.50%)	Gupta <i>et al</i> ^[53]
Ludhiana	(3.08%)	Wander <i>et al</i> ^[54]
South Indians		
Tamil Nadu	(14.30%)	Ramachandran <i>et al</i> ^[55]
Tamil Nadu	(11.00%)	Mohan <i>et al</i> ^[56]
Migrant Indians		
London, United Kingdom	(17.00%)	Bahl <i>et al</i> ^[57]
Illinois, United States	(10.00%)	Enas <i>et al</i> ^[5]

This complexity also contributes to difficulties in diagnosis and prognosis of the disease. Diagnostic difficulties also hamper the optimal and personalised treatment for patients. In recent years the role of genetic variability on the development of CAD has been extensively been studied^[1,2] which is impacting upon our understanding of phenotypic outcomes and clinical complications. New developments in genomics, epigenomics and exposomics (environmental risk factors across the life span) would result into the improved understanding of the different phenotypes observed in CAD and would help in the better regimen of treatment. In the last century, there has been rapid increases in the global prevalence of CAD, which has become the important cause of cardiovascular mortality all over the world, is > 4.5 million deaths in the developing countries. By 2020, it is predictable that CAD will be the major source of disease burden universally^[2]. The prevalence of CAD varies in different ethnic groups which may show higher/lower genetic and environmental susceptibilities. India has also witnessed consistent increases in the prevalence of CAD over the past few decades and could become the number one killer if appropriate interventions are not planned and implemented. In Table 1 the incidence of CAD is shown in different parts of India.

It has been reported that CAD is increasing in a linear fashion as it has increased from 4% in 1960 to 11% in 2001 *i.e.*, almost every 25th individual in 1960 was having CAD, while in 2001 every 9th individual was having CAD. The CAD is declining internationally among Indians settled abroad, whereas, these rates are growing in the Indian subcontinent. Presently, 10%-12% of metropolitan Indians have CAD compared to 3% of the United States population. Many studies document that Asian Indians are at 3-4 times greater risk of CAD than white Americans/Europeans, 6 times higher than Chinese, and almost 20 times higher than Japanese^[3-7]. CAD prevalence has increased from 3.5% in the 1960s to 9.5% in the 1990s in urban populations of India^[8]. Current studies recognized occurrence of CAD to be 13.9% in the urban south Indians, 9.6% in urban north Indians^[9-11]. In 1990s, 33% of

cardiovascular deaths have been reported from India^[12] and it is likely that deaths from non-communicable diseases such as CAD will increase two times higher *i.e.*, 4.5 million in 1998 to 8 million in 2020 in India^[13].

As CAD has a multifactorial nature and the occurrence of the familial clustering in CAD led investigators to start searching for susceptibility genes. In Figure 1 different risk factors involved in the causation of CAD are summarized.

It is vital to keep in mind that certain genes may show population specific effects. There are hundreds of genes known to have functional allelic variations that may be important for determining an individual's vulnerability to CAD. There is much argument on results of published epidemiological studies until now. The differences may be due to differences in the techniques used or the population used to calculate the incidence, prevalence and other risks. It has been proposed that if multiple markers are used for assigning the risk, the results would be more conclusive clinically. Most important reason of concern in developing countries like India is the incomplete detection, treatment and control of CAD risk factors. The benefits of addressing the root cause of CAD, such as inflammation, smoking and cholesterol, together with preventive methodology will be useful in improving quality of life and saving lives. This in turn may be translated into preventive approaches to help reduce the risk of CAD using genetic and epigenetic approaches. Although CAD mortality in the Indians is highest than other populations^[14,15], the reason for increased risk, which has been recorded in both the Asian immigrants and among Indians in urban India; are not yet clear hence more systematic and comprehensive studies are required to understand the spectrum of genetic and epigenetic influences on CAD.

GENETIC BASIS OF CAD

Atherosclerosis involves multiple factors, hence understanding the genetic and environmental basis of this complex disease requires holistic approaches^[16-18]. A range of candidate genes (*e.g.*, *APOE*, *APOB*, *LPL*, *iNOS*, *ACE*, *COX2*, *CD14*, *P-Selectin*, *E-Selectin*, *MTHFR*, *PON1*, *TNF α*) have been investigated in relation to initiation, development and progression of CAD^[16-18]. A large number of studies using of candidate genes and genome-wide association analyses have shown some promising signals, but only a few have been confirmed to some extent which may be playing a role in CAD.

There are very few examples where single genes have played a role in causing atherosclerosis^[19,20]. Mostly, CAD is caused by the environmental factors however the risk increases when some risk associated genes are also present. Research on identical twins consistently shows significant genetic effect in the development of CAD or its risk factors (Table 2). Heritability for CHD vary from 40% to 60%^[21,22], suggesting a strong role of genes in the development of the disease. A detailed analysis of the many known CAD susceptibility genes and studies is be-

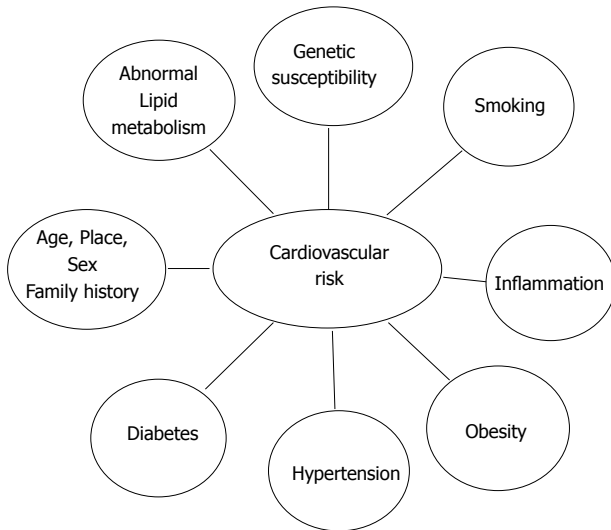


Figure 1 Cardiovascular risk factors.

yond the scope of this overview. This overview will focus on selected candidate genes in the *ApoA1-CIII-AIV* gene region.

SINGLE GENE DISORDERS AND CAD

Familial hypercholesterolemia

Familial hypercholesterolemia (FH) is a classic genetic disease in which increased cholesterol, tendon xanthomas, and early heart disease segregates together. Joseph Goldstein and Michael Brown showed that FH results from mutations in the low-density lipoprotein (LDL) receptor, which leads to impaired binding, internalization and degradation of LDL. Dose dependent relationship was observed, homozygotes patients had higher levels of cholesterol (> 600 mg/dL), whereas heterozygotes had levels of approximately 400 mg/dL. This variable penetrance is modified by genes and other risk factors such as diet, smoking, and physical activity level^[23]. Heterozygote frequency for this disease relatively high, approximately 1 in 500^[24] in most populations, however DNA screening and effective treatments are available now^[25,26].

Familial defective apolipoprotein B causing hypercholesterolemia

This comparatively common hypercholesterolemia (approximately 1 in 800), results from mutations in the major protein of LDL called Apolipoprotein B (ApoB). The mutations in ApoB prevent LDL binding to the LDL receptor. The majority of patients of this disorder carry a dominant mutation (codon 3500) and have lower cholesterol levels compared to FH patients. Other single-gene CHD/CAD traits are rare and of lower clinical/population significance^[20].

CANDIDATE GENES AND CAD

During last 30 years, there have been many advancements

Table 2 Genetic and environmental risk factors for coronary heart disease

Risk factors with a significant genetic component (heritability)

Elevated LDL and VLDL cholesterol (40%-60%)
 Low HDL cholesterol (45%-75%)
 Elevated triglycerides (40%-80%)
 Increased body mass index (25%-60%)
 Elevated systolic blood pressure (50%-70%)
 Elevated diastolic blood pressure (50%-65%)
 Elevated lipoprotein(a) levels (90%)
 Elevated homocysteine levels (45%)
 Type 2 diabetes mellitus (40%-80%)
 Elevated fibrinogen (20%-50%)
 Elevated C-reactive protein (40%)
 Elevated homocysteine levels (45%)

Gender

Age

Family history

Environmental risk factors

Smoking

Diet

Exercise

Infection

Foetal environment

Air pollution (particulates)

Risk factors for coronary heart disease can be subdivided into those that are determined significantly by genetic differences and those that are largely environmental (Based on Lusis *et al*^[58] 2004). VLDL: Very low density lipoprotein; LDL: Low density lipoprotein; HDL: High density lipoprotein.

in molecular genetic technology, development of sophisticated statistical tools and analyses which have contributed to improvements in human genetic research. One of the early developments was positional cloning technique, which allowed genetic mapping of many Mendelian diseases and traits. However for complex diseases, which involve many genes and environmental influences, this technique did not provide any major insights into genetic basis. Majority of our understanding of the genetic basis of CAD/CHD has been gained from studies of "candidate genes," and more recently genome wide association (GWA) studies. These population based studies have provided further insights into genetic susceptibilities/contributions to complex diseases. Some examples of these are given below.

APOLIPOPROTEIN E AND APOA1-CIII-AIV-AIV GENE CLUSTER

Apolipoprotein E (ApoE) is one of the extensively studied genetic locus as it plays a pivotal role in lipid metabolism and mediates the uptake of chylomicron and very low-density lipoprotein (VLDL) remnants. Utermann and colleagues^[27] identified genetic polymorphism at ApoE locus and its association with cholesterol levels and type III hyperlipidemia. The polymorphism and its CAD associations have been replicated in many global populations. E3 allele is the most common (approximately 60%) followed by E4 allele (approximately 30%) and E2

(approximately 10) in world populations. E4 allele carriers have increased plasma cholesterol levels compared to E3 allele carriers while E2 carriers have decreased plasma cholesterol levels. The allelic variation at ApoE locus explains approximately 5% of the variation in cholesterol levels^[28]. Type III hyperlipidemia, a relatively rare phenotype, are homozygous for the E2 allele, but not all E2 homozygous individuals have this disorder^[29]. Therefore, genotype-phenotype relationships may require contribution of other genetic or environmental factors.

In addition to ApoE, there is now strong evidence that mutations in hepatic lipase influence the levels of high-density lipoprotein (HDL)^[30], and the ApoAI-CIII-AIV-AV locus contributes to plasma triglyceride levels^[31]. Many studies have shown that Lp(a) levels are strongly influenced by Apo(a) gene^[32]. In addition, both hepatic lipase and the ApoAI-CIII-AIV-AV cluster influence LDL particle size, which significantly contributes to CHD risk^[33]. However, taken together, these genetic differences only explain a small amount of variation in plasma lipids and CHD/CAD phenotypes.

Dyslipidemia, a metabolic disorder, caused due to the defects in the synthesis, processing and catabolism of lipoprotein particles. Increased total cholesterol (TC)^[34], triglyceride (TG)^[35], LDL cholesterol (LDL-C)^[36], and apolipoprotein (Apo)B^[37], together with lower levels of ApoA1^[37] and HDL cholesterol (HDL-C)^[38] have been found to increase coronary artery disease (CAD) risk. Epidemiological and clinical studies have documented that above genetic factors/polymorphisms play a significant role in dyslipidemia^[39] susceptibilities along with environmental factors. Twin and family studies suggest there are considerable genetic contributions in the inter-individual variation in plasma lipid phenotypes with the heritability estimates ranging from 40%-60%^[40]. It has been suggested that understanding variation at these loci along with other newer genetic loci will provide a better understanding of the disease processes and contribution to personalized medicine.

ApoA1, is the main protein component of HDL-C, it functions in the activation of lecithin: cholesterol acyl-transferase, and facilitates the reverse cholesterol transport from peripheral tissues^[41]. ApoC3, is a 79-amino-acid protein formed mainly in the liver, is one of the major component of chylomicrons and VLDL and a minor component of HDL. ApoC3 prevents lipoprotein lipase and plays a key role in the catabolism of TG-rich lipoproteins. ApoA5 is detectable in very low-density lipoprotein, HDL, and chylomicrons and its concentrations are low compared to other apolipoproteins. Human *ApoA1/C3/A5* genes resides in the *ApoA1/C3/A4/A5* gene cluster on chromosome 11q23-q24^[42-45]. The *ApoA1/C3/A4/A5* gene cluster has emerged as a significant risk factor for hypertriglyceridemia and atherosclerosis^[41,42]. A number of studies have shown significant associations between single nucleotide polymorphisms (SNPs) in the *ApoA1/C3/A4/A5* gene cluster and raised plasma or serum lipid levels in humans, while others have

reported negative or inconsistent results^[42-46]. In addition there are many other SNPs involved in the inflammation and cell signalling with CAD and/or MI, some of these are summarized in Table 3.

One of the limitations of case control studies is that many false positive or false negative associations may emerge between different genetic markers and complex diseases like CAD. The reason for such results are: (1) controls are not properly selected; (2) sample size of both controls and cases because of which accurate power of the study is not generated and replication of results is not possible; and (3) position of single-nucleotide polymorphisms (SNP's) in terms of their effect on transcription of gene or protein expression. In general, results of small sample size studies (200-300 patients and control subjects) should be interpreted with caution and should be replicated with larger sample sizes. It is important to confirm that genotype distributions are not skewed, especially in the control group. Large deviations from the Hardy-Weinberg equilibrium, may suggest that the control group is not necessarily the representative of healthy and randomly sampled individuals. This departure may also highlight issues with genotype scoring. Recent genome-wide sequencing research has revealed extensive level of variation and heterogeneity between individuals and populations, which should be considered when choosing SNPs and interpreting SNP data. Some of the early SNP association studies failed to include the effect of the polymorphism on gene expression or protein function and genotype-phenotype correlations. This information could reveal if an SNP is the actual cause or solely a marker which may be in linkage disequilibrium another causal variant. These analyses could provide significant clues for understanding the pathophysiologic mechanisms behind clinical outcomes. It is important to correct/control for the age, gender, ethnicity, and other confounders in heart disease genetic association studies. There should be a holistic approach to understand the role of genes, environment and life style factors in CAD susceptibilities and progression.

Recently, genetic analyses have expanded to whole genome sequence analysis and genome-wide association studies (GWAS) as these analyses eliminates biases in the selection of the candidate genes. A number of GWAS studies have identified new loci in previously unsuspected genomic regions. These analyses have shown, novel biological pathways involved in the disease states and development of novel therapies. Many recent studies have shown only limited evidences may exist where the genetic variants may be associated with MI or only with CAD. A care has to be taken in interpreting the GWAS data as large number of variant alleles may be found but one should consider only elegant systems genetics approach to Plaisier *et al*^[47] used similar approach and found that *FADS3* is a causal gene for familial combined hyperlipidemia (*FCHL*) and elevated triglycerides in Mexicans. The authors used network gene co-expression analysis and SNP data to assign a function to the genetic variants

Table 3 Example of association studies of factors involved in inflammation and cell signalling with coronary artery disease and/or myocardial infarction

Gene	Polymorphism	Ref.	Suggested results
CRP	1059G/C	Zee <i>et al</i> ^[59]	No significant association with non-fatal MI, stroke or cardiovascular death
ICAM-1	Lys-469-glu	Jiang <i>et al</i> ^[60]	Association with MI and CAD
E-selectin	Ser-128-Arg, Leu-554-phe, G98T	Wenzel <i>et al</i> ^[61]	Associated with angiographic proof of severe CAD in patients < 50 yr
	Ser-128-Arg, G98T	Herrmann <i>et al</i> ^[62] Zheng <i>et al</i> ^[63]	No association with MI T allele more common in younger patients with angiographic CAD
P-selectin	Ser-128-Arg Pro715	Ye <i>et al</i> ^[64] Herrmann <i>et al</i> ^[65] , Kee <i>et al</i> ^[66]	Association with early-onset CAD Possibly has a protective role from MI
	S290N, N562D, V599L, T715P, T741T	Tregouet <i>et al</i> ^[67]	Protective effect of the P715; S290N and N562D associated with MI, when carried by certain haplotype
TNF- α and β	C-2123G, A-1969G, Thr715Pro -863C/A, -308G/A (TNF- α), 252G/A (TNF- β)	Barbaux <i>et al</i> ^[68] Koch <i>et al</i> ^[69]	Polymorphisms associated with P-selectin levels but not with MI No association of TNF or IL-10 polymorphisms with MI or CAD
TNF- α	Five polymorphisms	Herrmann <i>et al</i> ^[65]	No association to MI or CAD
TNF- α and β	TNF- α 308 G/A, TNF- β 252 A/G	Padovani <i>et al</i> ^[70]	No association to MI
TNF- α and β	TNF- β 308 G/A, TNF- β 252 A/G	Keso <i>et al</i> ^[71]	No association to old MI by autopsy or CAD
TNF- α	308 G/A	Francis <i>et al</i> ^[72]	No association to angiographic CAD
IL-1 cluster	IL-1 α (-889), IL-1b (-511), IL-1 β (+3953), IL-1RA intron 2 VNTR	Francis <i>et al</i> ^[72]	No association to angiographic CAD IL-1RA VNTR allele 2 associated with single-vessel CAD
IL-1-RA	IL-1RA intron 2 VNTR	Manzoli <i>et al</i> ^[73]	No clear-cut association to CAD or MI
IL-1 cluster	IL-1 β 511 C/T, IL-1RA intron 2 VNTR	Vohnout <i>et al</i> ^[74]	No association to angiographic CAD with either polymorphisms
IL-1-RA	IL 1RN-VNTR	Zee <i>et al</i> ^[75]	No association with risk for future MI
IL-1 β , IL-RA	IL-1 β 511 C/T, IL-1RA intron 2 VNTR	Momiyama <i>et al</i> ^[76]	IL-1 β (-511)C/C and IL-1Ra (intron 2)2- or 3- repeat allele both associated with CAD, association with MI only in patient who are seropositive for Chlamydia pneumoniae
IL-6	IL-6 G (-174)C promoter polymorphism -174 (G/C), -572 (G/C), -596 (G/A), +528 I/D	Nauck <i>et al</i> ^[77] Georges <i>et al</i> ^[78]	No association with the risk for CAD or MI -174 C associated with MI (OR = 1.34)-174 C more frequent in patients with two or fewer stenosed vessels than in patients with three vessel lesions
IL-10	3 IL-10 promotor polymorphisms (1082G/A, -819C/T and -592C/A)	Koch <i>et al</i> ^[69]	No association with MI or CAD
TGF- β_1	7 polymorphisms	Donger <i>et al</i> ^[79]	No association with risk for MI
	29 T/C	Yokota <i>et al</i> ^[80]	T allele is a risk factor for MI in middle-aged Japanese men
	-509T	Wang <i>et al</i> ^[81]	No association with CAD
Stromelysin (MMP-3)	7 polymorphisms	Cambien <i>et al</i> ^[82]	No association with degree of angiographic CAD, Pro25 allele associated with MI in some regions.
	5 polymorphisms	Syrris <i>et al</i> ^[83]	No association of either polymorphisms with CAD
	5A-117/6A promoter polymorphism (5A/6A)	Schwarz <i>et al</i> ^[84]	No association with the risk for MI, 6A allele marker for progression of CAD
	5A/6A	Terashima <i>et al</i> ^[85]	5A allele associated with risk for MI
	5A/6A	Kim <i>et al</i> ^[86]	5A allele associated with stable angina
PECAM-1 (CD31)	5A/6A	Humphries <i>et al</i> ^[87]	6A genotypes at greater risk for CAD related events in nonsmokers, 5A/5A genotypes amplifies risk in smokers
	5A/6A		Homozygosis for 6A associated with greater progression of angiographic CAD
	5A/6A	Ye <i>et al</i> ^[88]	
PECAM-1 (CD31)	Val 125Leu, Asn563Ser and Gly670Arg	Sasaoka <i>et al</i> ^[89]	563Ser/Ser and 670Arg/Arg genotypes associated with MI
	Val 125Leu, Asn563Ser	Wenzel <i>et al</i> ^[90]	125 Val and 563Asn associated with early onset of CAD (< 50 yr)
	Leu 125Val, Ser563Asn	Song <i>et al</i> ^[91]	125Val and 563Asn associated with CAD
	Val125Leu	Gardemann <i>et al</i> ^[92]	No association with MI; weak association of Val125 with CAD in low-risk patients without HTN or DM (OR = 1.54; 95%CI: 1.03-2.3)

CRP: C-reactive protein; DM: Diabetes mellitus; HTN: Hypertension; ICAM: Intercellular adhesion molecule; IL: Interleukin; MI: Myocardial infarction; MMP: Matrix metalloproteinase; PECAM: Platelet endothelial cell adhesion molecule; TGF: Transforming growth factor; TNF: Tumor necrosis factor; VNTR: Variable number of tandem repeats.

rs3737787 (1q21-q23) in *USF1* gene, which was previously identified to be associated with *FCHL*. It is envisaged that new methods like Network medicine^[48] will play an important role in these analyses and the advancement of our understanding of pathophysiological mechanisms

of diseases like CAD and MI.

CONCLUSION

This overview has highlighted some of the important

challenges regarding the use of genetic approaches to investigate complex diseases. The recent research using genomic, epigenomics and exposomic approaches is providing a range of patient centric tools which will help better classification of phenotypes and personalised medicine for CAD patients. The mechanisms underlying the association of these loci to CAD/MI remain largely unknown and the effects are relatively small. Hence the future challenges are (1) discovering new genetic variants through large-scale meta-analyses, using pathway-based approaches, and high throughput sequencing; (2) illustrating the mechanisms for the identified loci to CAD; and (3) translating the findings from CAD- GWASs and epigenetic analyses to novel and optimized therapeutic strategies.

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WJC 6th Anniversary Special Issues (3): Cardiomyopathy

Hypertrophic cardiomyopathy: Can the noninvasive diagnostic testing identify high risk patients?

Li Zhang, Obinna Mmagu, Liwen Liu, Dayuan Li, Yuxin Fan, Adrian Baranchuk, Peter R Kowey

Li Zhang, Obinna Mmagu, Peter R Kowey, Center for Clinical Cardiology, Lankenau Institute for Medical Research, Lankenau Medical Center, Jefferson Medical College, Philadelphia, PA 19096, United States

Liwen Liu, Department of Ultrasound, Xijing Hospital, Forth Military Medical University, Xi'an 710032, Shaanxi Province, China

Dayuan Li, HeartEast Heart Care, HealthEast Care System, St. Paul, MN 55102, United States

Yuxin Fan, John Welsh Cardiovascular Diagnostic Laboratory, Department of Pediatrics-Cardiology, Baylor College of Medicine, Houston, TX 77030, United States

Adrian Baranchuk, Arrhythmia Service, Kingston General Hospital, Queen's University, K7L 2V7, Ontario, Canada

Author contributions: Zhang L, Mmagu O, Liu L, Li D and Fan Y performed literature search and contributed in manuscript writing; Baranchuk A and Kowey PR performed manuscript editing.

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Correspondence to: Li Zhang, MD, Associate Professor, Center for Clinical Cardiology, Lankenau Institute for Medical Research, Lankenau Medical Center, Jefferson Medical College, R129A, 100 Lancaster Avenue, Wynnewood, PA 19008, United States. ldlzhang@gmail.com

Telephone: +1-484-4762694 Fax: +1-484-4761658

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visualization of the left ventricular chamber, allowing precise localization of the distribution of hypertrophy and measurement of wall thickness and cardiac mass. Moreover, with late gadolinium enhancement, patchy myocardial fibrosis within the area of hypertrophy can be detected, which is also helpful in risk stratification. Genetic testing is encouraged in all cases, especially in those with a family history of HCM and SCD.

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Key words: Hypertrophic cardiomyopathy; Sudden cardiac death; Noninvasive diagnostic testing

Core tip: Hypertrophic cardiomyopathy (HCM) is the most common cause of sudden cardiac death (SCD) in the young, particularly among athletes. Noninvasive diagnostic testing is important for risk assessment. Extreme left ventricular hypertrophy, documented ventricular tachycardia and fibrillation increase the risk of SCD. Fragmented QRS complex and T wave inversion in multiple leads are more common in high risk patients. Cardiac magnetic resonance imaging with late gadolinium enhancement, patchy myocardial fibrosis within the area of hypertrophy can be detected, which is also helpful in risk stratification. Genetic testing is encouraged in all cases, especially in those with family history of HCM and SCD.

Abstract

Hypertrophic cardiomyopathy (HCM) is the most common cause of sudden cardiac death (SCD) in the young, particularly among athletes. Identifying high risk individuals is very important for SCD prevention. The purpose of this review is to stress that noninvasive diagnostic testing is important for risk assessment. Extreme left ventricular hypertrophy and documented ventricular tachycardia and fibrillation increase the risk of SCD. Fragmented QRS and T wave inversion in multiple leads are more common in high risk patients. Cardiac magnetic resonance imaging provides complete

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INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a common au-

tosomal dominant cardiac disease, affecting 1 in 500 people^[1]. Cardiomyocyte hypertrophy, disarray, fibrosis and ventricular wall thickening are the pathological hallmarks of HCM. Although the majority of affected individuals present with mild symptoms or are asymptomatic, HCM is the most common identifiable cause of premature sudden cardiac death (SCD) in the young, especially the young athlete^[1]. Since SCD can be the first manifestation in concealed cases and some symptomatic patients do bear a high risk of SCD, timely diagnosis and risk stratification for appropriate therapy and SCD prevention such as prophylactic implantable cardioverter-defibrillator (ICD) therapy are very important. The common risk factors associated with SCD are family history of HCM-related SCD, left ventricular wall thickness ≥ 30 mm, documented ventricular tachyarrhythmia such as frequent and/or prolonged bursts of non-sustained ventricular tachycardia (VT) and ventricular fibrillation (VF), as well as abnormal blood pressure response to exercise^[2].

The diagnosis of HCM is based on echocardiography and/or cardiac magnetic resonance images (CMRI), wherein a non-dilated, hypertrophied left ventricle is found in the absence of any other systemic or cardiac event that explains the specific pathology, mostly arterial hypertension^[3]. Since the outcome varies among affected individuals, the purpose of this review is to elaborate the usefulness of noninvasive diagnostic testing for identifying high risk patients with HCM.

ELECTROCARDIOGRAM

The electrocardiogram (ECG), the most basic test in cardiovascular disease management, is abnormal in the vast majority of patients diagnosed with HCM. In general, if patients meet the ECG criteria for left ventricular hypertrophy (LVH), the absence of an apparent cause should raise the suspicion of HCM^[4]. The history and physical examination may be negative, and the signs of LVH on an ECG may occur earlier than the increase in the thickness of left ventricular wall detected by echocardiography^[4,5]. Making a correct diagnosis in a timely manner is essential in SCD prevention.

QRS-ST-T changes in sinus rhythm

Presence of abnormal Q waves such as deep Q waves in multiple leads is common in patients with HCM^[6]. Abnormal Q waves may appear prior to the increased QRS amplitude^[7]. A deep Q wave is considered if the amplitude ≥ 3 mm or 1/4 of the R wave. In HCM deep Q waves are usually seen in more than two contiguous leads^[8]. There are differences in terms of the significance of deep Q waves between the young and adults^[6]. Presence of deep Q waves in children has yielded a higher specificity and sensitivity than adults in the diagnosis of HCM^[6]. The mechanisms of deep Q waves in HCM are: (1) the electrical inactivation due to myocardial fibrosis; and (2) the altered direction of resultant initial QRS vector due to increased electrical forces of disproportionate hypertrophy of the basal septal and/or ventricular free

wall, unopposed by apical forces^[9]. Presence of deep Q waves in multiple leads is thought to be associated with an increased incidence of SCD^[9].

Increased QRS amplitude is the most common ECG abnormality in HCM. It has been reported that increased QRS amplitude in the limb leads increases the likelihood of SCD in both children and adults with HCM^[10]. Increased QRS duration (QRSD) is seen in septal and concentric HCM patients^[11]. Ostman-Smith *et al.*^[10] measured QRS amplitude and duration in HCM subjects with and without cardiac arrest and SCD. They found that increased QRS amplitude-duration product is a better indicator of high risk HCM patients. Among the high risk patients that have undergone ICD therapy, there is a positive correlation between increased QRSD and defibrillation thresholds^[12].

It is known that fragmented QRS complex (fQRS) in multiple ECG leads is associated with myocardial scarring or fibrosis in ischemic and non-ischemic cardiomyopathies. In the latter, patchy fibrosis are located in mid-myocardium or sub-epicardium, and predominantly in the perivalvular areas. Femenía *et al.*^[13] reported a female patient with recurrent syncope diagnosed with HCM at age 9 and had an ICD placed at age 16 after aborted SCD due to VF. Her ECG at age 16 showed fQRS in 12 leads. During a 2-year follow-up, this patient presented with sustained VT requiring anti-tachycardia pacing and ICD shocks^[13]. In a large sample study, they found that fQRS located in the lateral area increases the likelihood of ICD therapy^[14]. Therefore they postulated fQRS should be incorporated in multivariate models for SCD prediction, along with more classical risk factors^[13,14].

ST segment elevation in HCM is viewed as a marker of disease progression^[15]. Furuki *et al.*^[15] found a close correlation between convex ST elevation and left ventricular enlargement and wall motion abnormalities with a specificity of 85% and a sensitivity of 62%, respectively.

Ostman-Smith *et al.*^[10] found that HCM patients with high risk for SCD had negative T waves in the limb and precordial leads. Moreover, negative T waves in the precordial leads has a positive correlation with the extent of LVH in HCM^[7]. On echocardiography, the maximum wall thickness was 19.2 ± 5.2 mm with negative T waves compared to 13.5 ± 5.1 mm without negative T waves^[7]. Microvolt-T wave alternans (TWA), a surrogate for unstable ventricular repolarization properties, have been associated with an increased likelihood of VT/VF^[16]. Momiyama *et al.*^[16] also demonstrated that among 7/17 HCM patients classified as high-risk individuals, only two of them did not show TWA.

Ventricular arrhythmia

Documented VT and/or VF are direct risk factors for SCD^[17]. In HCM, VF may occur without the preceding VT. A study by Cha *et al.*^[18] revealed that sinus tachycardia or atrial fibrillation were the most common rhythms that initiated sustained VT followed by ICD discharges in high risk patients. Sustained VT is common in symptomatic individuals^[19]. Medeiros *et al.*^[20] noted that the arrhyth-

mias with the highest prevalence according to their ICD storage recordings were sustained VT and VF.

Recurrent or repetitive non-sustained VT (>10 beats) is considered a risk for SCD in HCM^[21]. On ambulatory ECG monitoring, non-sustained VT occurs in about 25% of HCM patients^[22]. Gimeno *et al*^[22] showed that exercise-induced non-sustained VT was associated with a 3.73 fold rise in SCD. 2D speckle tracking has also been used as an important tool to predict non-sustained VT in HCM patients^[23]. According to one study, the results obtained by 2D speckle tracking are similar to the results obtained by ambulatory Holter ECG^[23]. There have also been reports of the incidence of non-sustained VT by provocative maneuvers such as Valsalva^[24]. Increased vagal tone has been considered a potential mechanism for the occurrence of non-sustained VT^[24,25]. The current recommendation is consideration of ICD placement, even if non-sustained VT is the only risk factor^[21].

ECHOCARDIOGRAPHY

Echocardiography is an integral diagnostic modality for HCM because it is highly reproducible and cost effective. LVH, the most important phenotypic characteristic of HCM, can be easily revealed by echocardiography. The extent of left ventricular wall thickness is associated with an increased risk of SCD. Maximum left ventricular wall thickness ≥ 30 mm is termed extreme left ventricular hypertrophy, and is an independent predictor of SCD in the young^[26-28]. Spirito *et al*^[26] observed 480 cases of HCM consecutively for an average follow-up of 6.5 years. Patients were divided into five groups according to the maximum left ventricular wall thickness: ≤ 15 mm, 16-19 mm, 20-24 mm, 25-29 mm, and ≥ 30 mm, respectively. They found that the 20-year cumulative risk for SCD was up to 40% in the group with left ventricular wall thickness ≥ 30 mm. Patients with extreme left ventricular hypertrophy were mostly young, with only mild symptoms or with no symptoms at all. Neither did they have any evidence of left ventricular outflow tract obstruction. Thus the authors suggest that young patients with extreme left ventricular hypertrophy (≥ 30 mm), should consider prophylactic implantation of ICD regardless of the presence or absence of other risk factors. Elliott *et al*^[27] found that in HCM patients with left ventricular wall thickness, the relative risk (RR) increased by 1.31 (95%CI: 1.03-1.66) for each additional 5 mm. In HCM microvascular dysfunction, cardiomyocyte hypertrophy and disarray can lead to myocardial ischemia and fibrosis^[29]. The latter is a substrate for reentrant tachyarrhythmia and SCD^[30]. HCM patients with extreme left ventricular hypertrophy indeed bear a higher risk of SCD and more frequent ICD discharges. Nevertheless, it does not necessarily mean that patients with left ventricular wall thickness < 30 mm are considered low risk. In the later stages of the disease when the left ventricular ejection fraction (LVEF) may be below 50% with left ventricular wall thinning, apical aneurysm and ventricular chamber dilatation^[31], the risks of SCD and all-cause

mortality increase^[31,32].

CARDIAC CT

Although some of the newer systems are safe for ICD patients, MRI in general is hazardous to patients with implanted devices. As an alternative, Shiozaki *et al*^[33] examined the value of delayed enhancement multidetector computed tomography (MDCT). They showed that myocardial fibrosis was found in 96.4% of patients with ICD using MDCT^[33]. However, it must be noted that ICD cables caused artifacts and may have overrepresented the findings of myocardial fibrosis in these patients^[33].

CMRI

Although echocardiography plays a central role in the assessment of HCM, it is sometimes limited by poor acoustic windows, incomplete visualization of the left ventricular wall, and inaccurate evaluation of left ventricular mass^[34]. With excellent spatial resolution and border definition, CMRI provides complete visualization of the left ventricular chamber, allowing precise localization of the distribution of hypertrophy and measurement of wall thickness and cardiac mass (Figure 1). CMRI is superior to echocardiography for the detection of apical and focal basal anteroseptal variants and in recognizing noncontiguous areas of HCM^[35]. CMR cine imaging provides evaluation of cardiac morphological information including systolic anterior motion of the anterior mitral leaflet with dynamic outflow tract obstruction, mitral regurgitation, apical aneurysms, and papillary muscle abnormalities. CMR stress perfusion imaging can identify areas of microvascular dysfunction or mismatch between left ventricular mass and coronary flow.

Based on CMRI findings in patients with HCM, distribution and extent of LVH is variable including asymmetrical septal, apical, localized, or concentric hypertrophy, but these usually are not extensive. Basal anterior left ventricular free wall and the contiguous anterior ventricular septum are the most commonly hypertrophied segments^[34]. LVH can be focal (1-2 segments), intermediate (3-7 segments), or diffuse (> 8 segments). The number of hypertrophied segments is greater in patients with left ventricular outflow tract obstruction than without and was associated with an advanced New York Heart Association functional class. Left ventricular wall thickness was greater in segments with late gadolinium enhancement (LGE) than without. Segmental left ventricular hypertrophy largely confined to the anterolateral free wall, posterior septum, or apex were underestimated or undetected by echocardiography. These observations support an emerging role for CMR in the contemporary evaluation of patients with HCM.

Moreover, LGE plays a critical role in risk stratifying HCM patients (Figure 1). Myocardial fibrosis is present in up to 80% of patients with HCM, with a characteristic patchy pattern of LGE generally occurring in areas of hypertrophy^[34]. In addition, the extent of fibrosis has been shown to correlate positively with regional hypertro-

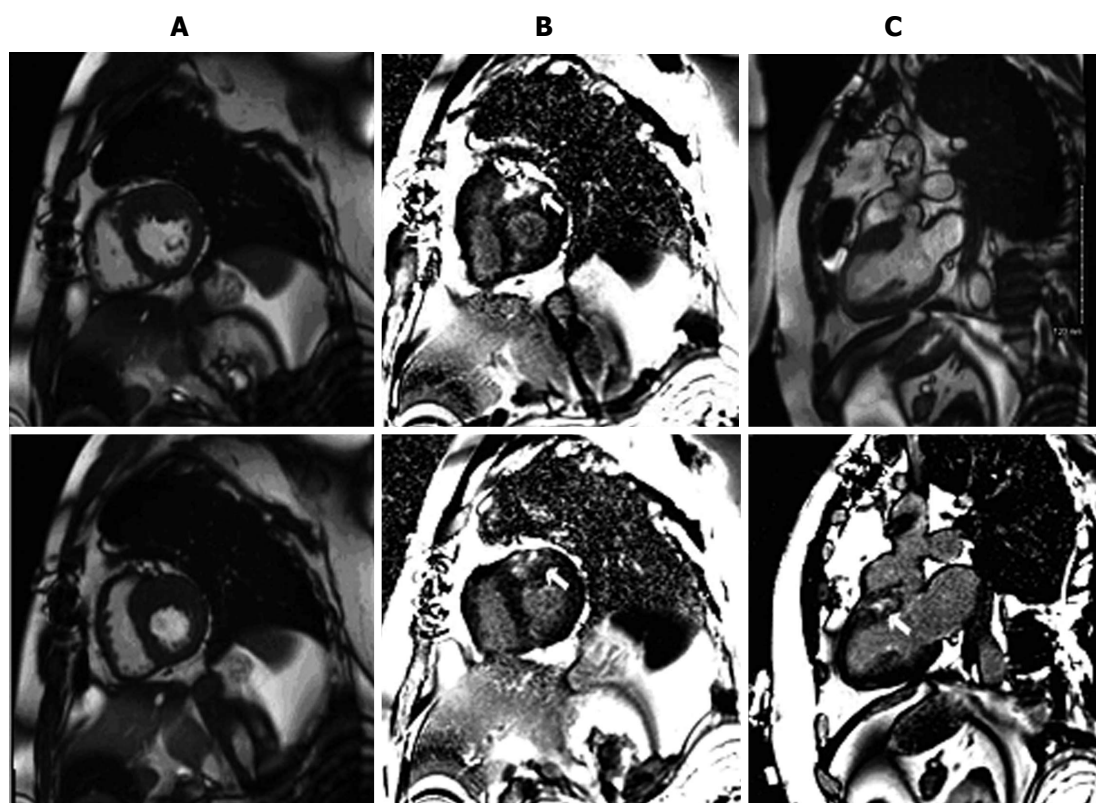


Figure 1 Basal anterior hypertrophic cardiomyopathy in a 45-year-old man with a history of syncope. Cardiac magnetic resonance imaging demonstrated severe thickening of basal anterior wall with a maximal measurement of 25 mm. A: Two chamber short-axis cine images; B: Late delayed gadolinium enhanced images; C: Two chamber long-axis cine images (top panel). Late delayed gadolinium enhanced images (bottom panel). Patchy, non-coronary artery disease scarring in the hypertrophied areas is indicated by white arrows.

phy and inversely with regional contraction. Consistently, in another clinical study^[36] with 243 consecutive HCM patients, the presence of scar was an independent predictor of death, with an odds ratio of 5.47 for all-cause mortality and of 8.01 for cardiac mortality. Similarly, the risk of unplanned heart failure admissions, deterioration to NYHA functional class III or IV, or heart failure-related death has been shown to be statistically greater in those with fibrosis^[37]. In a study with 424 HCM patients^[38], LGE-positive patients were more likely to have episodes of non-sustained VT, more episodes of non-sustained VT per patient, and a higher frequency of ventricular extrasystoles per 24 h, with all cases of SCD and appropriate ICD discharges occurring in LGE-positive patients. More recently, a meta-analysis^[39] of four studies evaluating 1063 HCM patients over an average follow-up of 3.1 years demonstrated that there are significant relationships between LGE and cardiovascular mortality, heart failure death, and all-cause mortality in HCM patients. Additionally, LGE and SCD/aborted SCD displayed a trend toward significance. The assessment of LGE in HCM patients by CMRI has the potential to provide important information to improve risk stratification in clinical practice.

ROLE OF GENETIC TESTING

Since the pathogenic missense mutation in the β -myosin heavy chain gene (MYH7 R403Q) was revealed two

decades ago, > 1400 mutations have been identified in putative HCM-susceptibility genes. The most common genetic subtype is sarcomeric-HCM, caused by mutations in genes encoding proteins in the myofilaments of the cardiac sarcomere^[40]. Among patients with positive genetic tests, MYBPC3 (myosin-binding protein C) and MYH7 are, by far, the two most common HCM-associated genes with an estimated prevalence of 25%-35% for each gene, while other genes including troponin T, troponin I, α -tropomyosin, and α -actin each account for a small proportion of patients (1% to 5%)^[41]. Collectively, the known causal genes account for about two-thirds of all HCM cases while one-third of the causal genes for HCM are yet to be identified^[42].

Genetic testing for HCM has been commercially available for almost a decade. However, the low mutation detection rate and cost have hindered uptake^[43]. Currently, genetic testing has been recommended for any patients with an established clinical diagnosis of HCM and for family members following the identification of the HCM-causative mutation in the index case^[44]. Multivariate analysis advocates this recommendation by identifying female gender, increased left-ventricular wall thickness, family history of hypertrophic cardiomyopathy, and family history of SCD as being associated with greatest chance of identifying a gene mutation^[43].

Including genetic testing in the diagnostic strategy is also more likely to be cost effective than clinical tests

alone when considering family screening and prevention of SCD^[45,46]. The results of genetic testing identifies mutation carriers who will benefit from regular clinical investigation or early discussion of ICD. On the other hand, the result of genetic testing also identifies relatives without the causal mutation, who can be released without the need for long-term follow-up^[47].

With rapid developments in genetic testing technology, a whole exome or a panel of HCM-related genes can now be tested by the next generation sequencing simultaneously, which provides an opportunity to detect multiple mutations in the same or different genes that are responsible for HCM. Emerging evidence documents that patients with HCM who carry more than one independent disease-causing gene mutation may be at a greater risk for severe disease expression and adverse outcome^[48-51], especially in the absence of other conventional risk factors^[52]. These observations support the emerging hypothesis that double (or compound) mutations detected by genetic testing may confer a gene dosage effect in HCM, thereby predisposing patients to adverse disease consequence^[52]. It is observed that multiple mutation carriers are more likely to have suffered an out-of-hospital cardiac arrest or SCD^[43]. For those patients who test positive for two or three mutations, frequent follow-up or early intervention may be required. Therefore, the integration of genetic testing into the current testing paradigm is likely to improve the general management of affected families.

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WJC 6th Anniversary Special Issues (3): Cardiomyopathy

Alcoholic cardiomyopathy

Gonzalo Guzzo-Merello, Marta Cobo-Marcos, Maria Gallego-Delgado, Pablo Garcia-Pavia

Gonzalo Guzzo-Merello, Marta Cobo-Marcos, Maria Gallego-Delgado, Pablo Garcia-Pavia, Heart Failure and Inherited Cardiac Diseases Unit, Department of Cardiology, Hospital Universitario Puerta de Hierro, 28222 Madrid, Spain

Author contributions: Guzzo-Merello G reviewed the literature, interpreted the data, and wrote the manuscript; Cobo-Marcos M, and Gallego-Delgado M interpreted the data and contributed to the writing of the manuscript; Garcia-Pavia P was involved in the drafting of the manuscript and revising it critically for important intellectual content; Guzzo-Merello G and Garcia-Pavia P are responsible for the overall content and take responsibility for the final submission.

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Correspondence to: Pablo Garcia-Pavia, MD, PhD, Department of Cardiology, Hospital Universitario Puerta de Hierro, Manuel de Falla, 2. Majadahonda, 28222 Madrid, Spain. pablogpavia@yahoo.es

Telephone: +34-911-917297 Fax: +34-911-917718

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Core tip: Cardiac dysfunction associated with excessive alcohol intake is a specific cardiac disease known as alcoholic cardiomyopathy. In spite of its clinical importance, data on alcoholic cardiomyopathy and how alcohol damages the heart are limited. In this review, we evaluate available evidence linking excessive alcohol consumption with heart failure and dilated cardiomyopathy. Additionally, we discuss the clinical presentation, prognosis and treatment of alcoholic cardiomyopathy.

Guzzo-Merello G, Cobo-Marcos M, Gallego-Delgado M, Garcia-Garcia P. Alcoholic cardiomyopathy. *World J Cardiol* 2014; 6(8): 771-781 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i8/771.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i8.771>

Abstract

Alcohol is the most frequently consumed toxic substance in the world. Low to moderate daily intake of alcohol has been shown to have beneficial effects on the cardiovascular system. In contrast, exposure to high levels of alcohol for a long period could lead to progressive cardiac dysfunction and heart failure. Cardiac dysfunction associated with chronic and excessive alcohol intake is a specific cardiac disease known as alcoholic cardiomyopathy (ACM). In spite of its clinical importance, data on ACM and how alcohol damages the heart are limited. In this review, we evaluate available evidence linking excessive alcohol consumption with heart failure and dilated cardiomyopathy. Additionally, we discuss the clinical presentation, prognosis and treatment of ACM.

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INTRODUCTION

Daily consumption of low to moderate amounts of alcohol has beneficial effects on cardiovascular health among both ischemic and non-ischemic patients^[1-3]. In contrast, chronic and excessive alcohol consumption could lead to progressive cardiac dysfunction and heart failure (HF)^[3].

HF is most frequently related to the presence of arterial hypertension and ischemic cardiomyopathy^[4,5]. In younger individuals, however, where HF is less prevalent, a heterogeneous group of cardiac diseases, collectively known as cardiomyopathies, represent the leading cause of HF and heart transplant in the world^[6]. Among cardiomyopathies, the variety that most often leads to HF and the first cause of heart transplant among young patients is dilated cardiomyopathy (DCM)^[6]. DCM is defined as left ventricular systolic dysfunction and dilatation, which may or may not be associated with a similar right ventricular dysfunction. Excessive alcohol consump-

tion is prominent among the multiple aetiologies causing DCM and has been considered the major cause of non-ischemic DCM in Western countries^[7-12].

Despite the key clinical importance of alcohol as a cause of DCM, relatively few studies have investigated the effects of alcohol on the heart and the clinical characteristics of DCM caused by excessive alcohol consumption (known as alcoholic cardiomyopathy). Moreover, conflicting results are available regarding several factors related to alcoholic cardiomyopathy (ACM), such as the precise amount of alcohol necessary to cause the disease, whether the long-term prognosis of ACM is similar to that of other forms of DCM, or whether complete alcohol abstinence is necessary to improve clinical outcomes.

In this review, we evaluate the available evidence linking alcohol consumption with HF and DCM. We also discuss the clinical presentation, prognosis and treatment of ACM.

HISTORICAL PERSPECTIVE

The depressing effect of alcohol on the heart has been known for some time. Indeed, the first account of the possible harmful effects of alcohol specifically on heart muscle was reported in the latter half of the 19th century. Expressions referring to “the heart of a wine drinker in Tübingen” and particularly a “Munich beer heart” were used and known in Germany during this time^[13].

Bollinger, a pathologist in Munich in the late 19th century, was perhaps the first to suspect a possible link between excessive alcohol consumption and sudden death in young individuals, an occurrence that alarmed public opinion at the time. The diagnosis of the source of those deaths was found after performing autopsies and discovering the characteristic left ventricular dilatation and hypertrophy. The findings that led Bollinger to establish a causal relationship between alcohol consumption and these structural abnormalities were both of a clinical and an epidemiological nature. Thus, he identified that the incidence of alcohol-related DCM was much higher in Munich, where alcohol intake was greater, than in other German cities. Indeed, he found 42 cases of ACM from among 1500 autopsies performed in Munich, contrasting with a single case in Berlin from 809 hearts analysed. Also, he observed that these individuals often presented co-morbidities closely associated with alcohol consumption, including delirium tremens and cirrhosis of the liver, and that 22 of the 42 deceased individuals were regular patrons of beer houses in Munich, where they could drink from 6 to 12 L of beer per day^[13].

Later, in 1902, William McKenzie, in his treatise on arterial and venous pulse and heart movements, described the existence of individuals who, in association with alcohol consumption, developed an accelerated heart pulse or swelling and engorgement of the veins, and according to his experience they had a poor prognosis with progressive heart failure. In their autopsies, he described finding dilated cavities of the heart and fatty degeneration of the ventricular walls^[14].

Since those initial descriptions, reports on several isolated cases or in small series of patients with HF due to DCM and high alcohol intake have been published^[15-17]. Some of these papers have also described the recovery of LVEF in many subjects after a period of alcohol withdrawal^[15-17].

DEFINITION OF ALCOHOLIC CARDIOMYOPATHY

At present ACM is considered a specific disease both by the European Society of Cardiology (ESC) and by the American Heart Association (AHA)^[18,19]. In the ESC consensus document on the classification of cardiomyopathies, ACM is classified among the acquired forms of DCM^[19].

The diagnosis of ACM is usually one of exclusion in a patient with DCM with no identified cause and a long history of heavy alcohol abuse. According to most studies, the alcohol consumption required to establish a diagnosis of ACM is over 80 g per day during at least 5 years^[9-12].

AMOUNT OF ALCOHOL REQUIRED TO PRODUCE ACM

Data on the amount of alcohol consumption required to cause ACM are limited and controversial.

The first study, which specifically focused on the amount of alcohol necessary to cause ACM, was conducted by Koide *et al*^[20] in 1975. The authors examined the prevalence of cardiomegaly by means of chest x-rays and related it to alcohol consumption among a consecutive series of Japanese males of working age. They found that 2 of the 6 individuals (33%) whose alcohol consumption exceeded 125 mL/d had cardiomegaly. In contrast, an enlarged heart was found in only 1 of 25 subjects with moderate consumption (4%), in 6 of 105 very mild consumers (5.7%), and in 4.5% of non-drinking individuals.

A second set of studies that are quoted when addressing this topic are those conducted in individuals who started an alcohol withdrawal program^[21-24]. In these studies, the authors estimated the amount and chronicity of alcohol intake and subsequently related the figures to a number of echocardiographic measurements and parameters. Although all of the studies reported an increase in left ventricular mass and volume, it cannot generally be stated that they provided the alcohol consumption dosage required to cause ACM.

Askanas *et al*^[21] found a significant increase in the myocardial mass and of the pre-ejection periods in drinkers of over 12 oz of whisky (approximately 120 g of alcohol) compared to a control group of non-drinkers. However, no differences were found in these parameters between the sub-group of individuals who had been drinking for 5 to 14 years and the sub-group of individuals who had a drinking history of over 15 years. Kino *et al*^[22] found

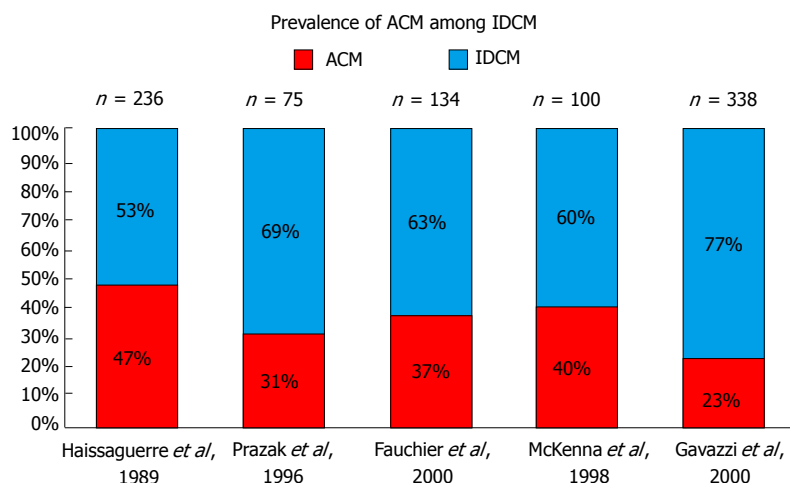


Figure 1 Prevalence of alcoholic cardiomyopathy among idiopathic dilated cardiomyopathy series. ACM: Alcoholic cardiomyopathy; IDCM: Idiopathic dilated cardiomyopathy.

increased ventricular thickness when consumption exceeded 75 mL/d (60 g) of ethanol, and the increase was higher among those subjects who consumed over 125 mL/d (100 g), without specifying the duration of consumption. In another study on this topic, Lazarević *et al*^[23] divided a cohort of 89 asymptomatic individuals whose consumption exceeded 80 g/d (8 standard units) into 3 groups according to the duration of their alcohol abuse. Subjects with a shorter period of alcohol abuse, from 5 to 10 years, had a significant increase in left ventricular diameter and volume compared to the control group. However, a systolic impairment was not found as the years of alcoholic abuse continued.

Unfortunately Lazarević *et al*^[23], as in most of these studies, systematically excluded patients with a history of heart disease or with HF symptoms. It is therefore possible that most of these studies may have also consistently omitted most alcohol abusers in whom alcohol had already caused significant ventricular dysfunction.

One of the exceptions in these accounts is the study conducted by Urbano-Márquez *et al*^[24], in which 46 asymptomatic alcohol abusers who were beginning an alcohol withdrawal program were studied together with 6 alcoholics identified at the emergency department due to HF symptoms. This is the only study describing the existence of a direct linear relationship between accumulated alcohol consumption throughout life and left ventricular mass ($r = 0.42$), fractional shortening ($r = 0.35$), and ejection fraction ($r = 0.46$) (all $P < 0.001$). A large number of studies, however, never reproduced this relationship, and it has been suggested that this relationship could correspond to the existence of a threshold dose above which the risk of suffering this disease increases^[25]. Kupari *et al*^[25], after reviewing the research by Urbano-Márquez, suggested a lifetime cumulative cut-off dose of alcohol of 20 kg/kg of weight. Actually, in the research by Urbano-Márquez *et al*^[24], slight dysfunction of the left ventricle had already appeared due to cumulative doses of 10 kg of alcohol per kg of weight.

Finally, it should be noted that a large majority of studies on the long-term prognosis of ACM used the cut-off point of 80 g/d for a minimum of 5 years to consider alcohol as the cause of DCM. Although this

figure may be sufficient to cause the structural alterations described above, we must stress that this value is arbitrary and is not based on robust experimental or epidemiological data; also, the average consumption of the individuals included in the research was always much greater^[9-12].

Additionally, the accepted ACM definition does not take into account a patient's sex or body mass index (BMI). As women typically have a lower BMI than men, a similar amount of alcohol would reach a woman's heart after consuming smaller quantities of alcohol.

EPIDEMIOLOGY OF ALCOHOLIC CARDIOMYOPATHY

For many decades, ACM has been considered one of the main causes of left ventricular dysfunction in developed countries. Specifically in the United States, ACM was declared the leading cause of non-ischemic DCM^[7]; a fact related to the high consumption of alcoholic beverages worldwide, which is particularly elevated in Western countries^[26].

Studies that have assessed the prevalence of ACM among IDCM patients have found high alcohol consumption in 3.8% to 47% of DCM patients. The lowest prevalence of ACM among DCM (3.8%) was obtained from a series of 673 patients admitted to hospital consecutively due to HF in the state of Maryland^[27]. This study included not only DCM, but also all causes of left ventricular dysfunction, including hypertensive heart disease, ischemic cardiomyopathy and heart valve disease. Furthermore, the inclusion criteria for ACM were very strict and required a minimum consumption of 8 oz of alcohol (200 g or 20 standard units) each day for over 6 mo. In contrast, European studies focusing on the prevalence of ACM included only subjects diagnosed with DCM and applied the consumption threshold of 80 g/d for ≥ 5 years, finding an ACM prevalence of 23%-47% among idiopathic DCM patients^[9-12] (Figure 1).

Finally, it should be noted that McKenna and co-workers, in one of the most frequently cited papers in the ACM field, reported an incidence of 40% in 100 individuals suffering from idiopathic DCM, but in this case

the consumption threshold used was only 30-40 g/d^[8].

EVIDENCE LINKING EXCESSIVE ALCOHOL CONSUMPTION AND DCM

The existence of a direct causal link between excessive alcohol consumption and the development of DCM is a controversial issue. While some consider that this toxin alone is able to cause such a disease^[18,19], others contend that it is just a trigger or an agent favouring DCM^[3,21,22].

At present, however, ACM is considered to be a disease in its own right^[18,19].

The evidence that allows this link to be established arises from 6 categories of research: (1) epidemiological studies; (2) experimental studies with controlled alcohol administration; (3) haemodynamic/echocardiographic studies analysing the effects generated by alcohol consumption on myocardial structure and function; (4) histological studies; (5) basic research studies identifying the mechanisms of alcohol-induced damage to the cardiomyocyte; and (6) studies analysing the positive clinical response to alcohol withdrawal.

Epidemiological studies

Epidemiological studies analysing the relationship between excessive alcohol consumption and the development of DCM have found the existence of a reciprocal link between both disorders.

In this respect, a higher prevalence of excessive alcohol consumption has been reported among individuals diagnosed with DCM than in the general population^[8].

In 1986, Komajda *et al.*^[28] reported that DCM patients admitted due to HF had higher alcohol consumption levels than patients admitted to undergo surgical procedures (101 mL/d *vs* 64 mL/d; RR = 7.6, $P < 0.001$).

Furthermore, Gillet published a similar study in which a cohort of 23 patients with DCM reported higher average daily alcohol consumption (82 g/d *vs* 30 g/d; $P < 0.001$) and a greater duration of that consumption (34 *vs* 22 years, $P < 0.001$) than a second group of 46 individuals suffering from other forms of heart disease^[29]. Also, in 1998 McKenna described an incidence of excessive alcohol consumption of 40% in a group of 100 DCM patients compared to 23% found in a control group of 211 healthy subjects^[8].

Furthermore, Fernández-Solá *et al.*^[30], when analysing a population of alcoholics, found a higher prevalence of DCM in alcoholics than among the general population. Specifically, among alcoholics they found a prevalence of DCM of 0.43% in women and 0.25% in men, whereas the described prevalence of DCM in the general population is 0.03% to 0.05%^[18,19].

Experimental studies

Experimental studies analysing the depressive properties of alcohol on the cardiac muscle invariably use similar approaches^[31-39]. Accordingly, a given amount of alcohol is administered to volunteers or alcoholics, followed by

the measurement of a number of haemodynamic parameters and, in some cases, echocardiographic parameters. Generally, following alcohol intake, healthy, non-drinking individuals showed an increase in cardiac output due to a decline in peripheral arterial resistance and an increase in cardiac frequency^[31]. However, during the time that these haemodynamic changes appeared, some researchers identified a possible decrease in the ejection fraction and other parameters related to systolic function^[32-39]. This was questioned by other authors, who pointed out that these conclusions could not be drawn, as alcohol itself also induces changes in the pre-load and after-load conditions, which influence cardiac contractility^[35]. However, in this context, experimental *in vitro* studies using cardiomyocytes have shown that alcohol depresses the contractile capacity of the myocardium, regardless of the sympathetic tone and the haemodynamic conditions^[36].

The capacity of alcohol to depress cardiac contractility became evident in studies carried out with chronic alcoholics and in patients with left ventricular dysfunction. In these patients, alcohol, in spite of causing vasodilatation and an increase in the heart rate, did not produce an increase in heart output or, if it did, it was lower than in healthy non-drinking individuals^[32,34]. Together, this suggests a depressed contractile capacity. This was specifically addressed by Regan, who found that, after an intake of 81 g of alcohol, the heartbeat volume of a group of chronic alcoholics was reduced and the end diastolic pressure increased, indicating that in these individuals there was a reduction in the left ventricular contractile reserve^[32]. This impairment of contractile capacity among chronic alcoholics was demonstrated in the same study using an after-load test with angiotensin. Results showed that the end diastolic pressure increased to a greater extent in alcoholics and was associated with a lower beat volume than in non-drinkers^[32].

Echocardiographic and haemodynamic studies in alcoholics

Myocardial impairment following chronic excessive alcohol intake has been evaluated using echocardiographic and haemodynamic measurements in a significant number of reports. In these studies, haemodynamic and echocardiographic parameters were measured in individuals starting an alcohol withdrawal program. The findings were analysed taking into account the amount and chronicity of intake and they were compared with the same parameters measured in a control group of non-drinkers.

The majority of the echocardiographic studies performed on asymptomatic alcoholics found only mild changes in their hearts with no clear impairment of the systolic function. For example, a slight increase in the pre-ejection period/left ventricular ejection time ratio (PEP/LVET) was found by some authors, suggesting a sub-clinical impairment of systolic function^[21,33]. Mathews and Kino found a small, but significant increase in left ventricular mass in individuals consuming at least 12 oz of whisky during 6 years and 60 g of ethanol per day, respectively^[22,40]. More recently, Lazarevic found a modest

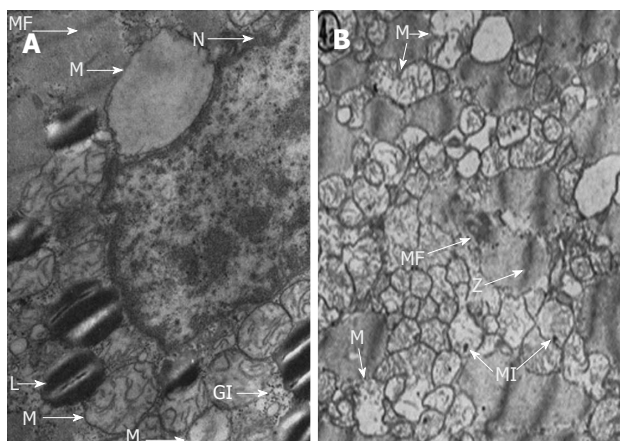


Figure 2 Cellular changes in alcoholic cardiomyopathy. L: Neutral lipids in the form of small cytoplasmic droplets; GI: Glycogen deposits; M: Mitochondria were swollen or oedema was present; N: Nucleus; MF: Myofibrils showed a progressively distorted structure (Z lines disrupted). Reproduced with permission from the American Heart Association^[42].

increase in end-systolic and diastolic left ventricular volumes and a subsequent thickening of the posterior wall in a cohort of alcoholics consuming at least 80 g during 5 years^[23]; however, no differences in systolic function were observed. Finally, only Urbano-Márquez *et al.*^[24] found a clear decrease in the ejection fraction, in a cohort of 52 alcoholics, which was directly proportional to the accumulated alcohol intake throughout the patients' lives.

Histological studies

Alterations caused by heavy alcohol intake have also been studied from the perspective of histopathology. Emmanuel Rubin analysed muscle biopsies from individuals who were previously non-drinkers and were submitted to a balanced diet with heavy alcohol intake during one month^[41]. Although no significant changes were found using conventional microscopy, when electron microscopy was employed he discovered intracellular swelling, glycogen and lipid accumulation, and alterations in the structure of the sarcoplasmic reticulum and of the mitochondria (Figure 2). These changes, though subtle, were similar to those found by Ferrans and Hibbs in eight deceased individuals diagnosed with ACM^[42,43]. On histological examination, various degrees of fibrosis, patchy areas of endocardial fibroelastosis, intramural blood clots and focal collections of swollen cells in both the epicardium and endocardium were found. Also, there were significant size variations in the myofibrils and they showed a relative decrease in the number of striations, in addition to swelling, vacuolisation and hyalinisation. Cell nuclei were larger than normal, morphologically difficult to define and they occasionally showed hyperpigmentation. The authors highlighted the presence of an extensive intracellular accumulation of neutral lipids, principally in the form of small cytoplasmic droplets. In a subsequent study using electron microscopy, the authors found histological features that could be superimposed onto those found in hearts that had suffered hypoxia, anoxia

or ischemia^[43]. Analogous to the sarcoplasmic reticulum, the mitochondria were swollen or oedema was present, with crest alterations and intra-mitochondrial inclusions suggesting degenerative processes (Figure 2). Moreover, myofibrils showed a progressively distorted structure, resulting in a homogeneous mass.

Despite these features, the structural changes do not seem to be specific, furthermore, they are not qualitatively different from those found in idiopathic DCM and they do not allow us to differentiate between the two conditions^[44]. It also appears that the changes emerging in ACM patients only differ from idiopathic DCM in quantitative terms, with histological changes being more striking in idiopathic DCM than in ACM^[44].

Basic studies on molecular mechanisms of myocardial damage

Basic research studies have described an abundance of mechanisms that could underscore the functional and structural alterations found in ACM. Because of this, their origin could be multifactorial and linked both to the alcohol molecule and to its main metabolite, acetaldehyde.

Coinciding with the histological studies mentioned above, the majority of research on molecular mechanisms describes dysfunctions of intracellular organelles prompting alterations in the lipid-energetic metabolism and in calcium homeostasis, which are especially relevant for the contractile activity of myofibrils.

In spite of numerous studies, the sequence of events that occur in alcohol-induced myocardial damage is still highly controversial. Although some authors contend that the initial event is the appearance of hypertrophy, the majority accept that the core event is the loss of cardiomyocytes.

The mechanisms described to date are shown in Figure 3 and they include: apoptosis^[45,46], alterations of the excitation-contraction coupling in cardiac myocytes^[47], structural and functional alterations of the mitochondria and sarcoplasmic reticulum^[41-43], changes in cytosolic calcium flows^[48], changes in calcium sensitivity of myofilaments^[49,50], alterations of mitochondrial oxidation^[37,38,46], deregulation of protein synthesis^[51-53], decrease of contractile proteins and disproportion between the different types of myofibrils^[54-56], changes in the regulation of myosin ATPase^[51], up-regulation of the L-type calcium channels^[57], increase of oxidative stress^[58,59], induction of ANP and p21 mRNA expression in ventricular myocardium^[45], and activation of the renin-angiotensin system and of the sympathetic nervous system^[60-62]. Additionally, it has been proposed that mechanisms of a genetic nature play a determining role in the pathophysiology of this disease.

The suspicion that there may be an individual susceptibility to this disease is underscored by the finding that only a small group of alcoholics develop ACM, and that a proportional relationship between myocardial damage and alcohol intake has not been proven.

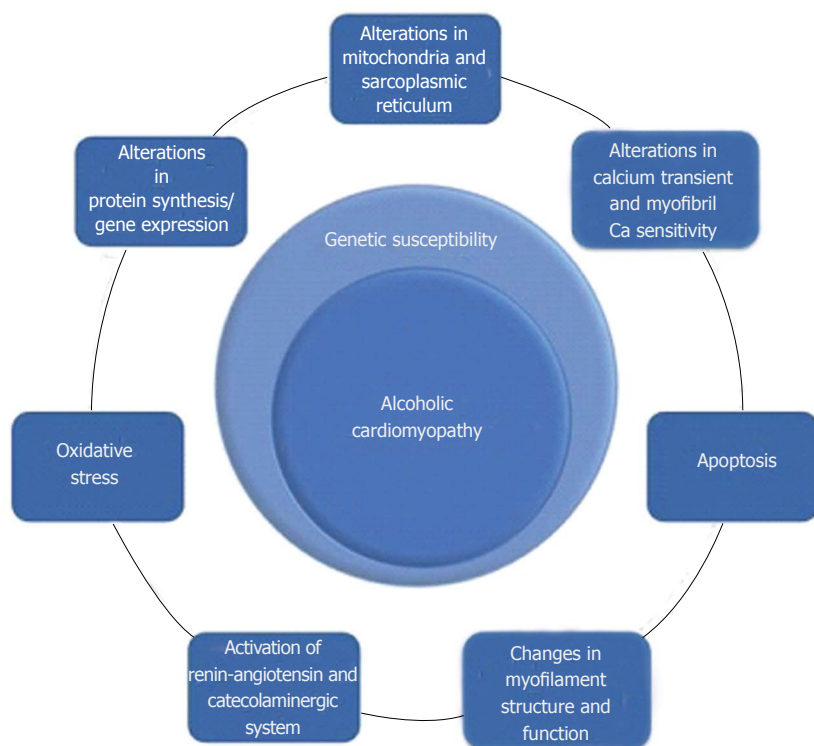


Figure 3 Alcoholic Cardiomyopathy. Pathophysiology.

Although some studies have detailed structural and functional damage in proportion to the amount of alcohol consumed during a patient's lifetime^[24], a large majority of authors have discarded this theory^[21-23,25]. Both the absence of a direct correlation and the theory of the existence of a threshold dose (above which some alcoholics develop ACM) require the presence of individual susceptibility to alcohol induced cardiac damage^[63]. It is unknown whether individual susceptibility would be related to increased vulnerability at the myocardial level and/or to impaired alcohol metabolism.

One of the few papers analysing genetic susceptibility in ACM was published by Fernández-Solà *et al*^[64] in 2002. He compared the prevalence of different polymorphisms of the angiotensin-converting enzyme gene in 30 ACM patients and in 27 alcoholics with normal ventricular function. The DD genotype was more frequent among ACM patients (56% *vs* 8%). Furthermore, 89% of the alcoholics with a DD genotype developed ACM, whereas only 13% of those with an II or ID genotype developed this condition. However, this individual susceptibility mediated by polymorphisms of the angiotensin-converting enzyme gene does not appear to be specific to ACM insofar as several diseases, including some that are not of a cardiologic origin, have been related to this genetic finding^[65].

Regarding individual susceptibility based on alcohol metabolism, data are scarce, but provocative findings arose from a study published in 2002 which showed that the cardio-depressive power of alcohol in mice varied according to the activity of the enzymes involved in the metabolism of alcohol^[66]. In this study, alcohol caused greater cardiomyocyte impairment in mice genetically modified with higher alcohol dehydrogenase activity. The mechanism by which cardiac damage occurred was not

fully elucidated, but it was proposed that it was due to the accumulation of acetaldehyde. Furthermore, mice that received an aldehyde dehydrogenase inhibitor experienced an additional impairment in contractility^[66]. Regrettably, the role of gene mutations in alcohol or aldehyde dehydrogenase and genetic polymorphisms including ADH1B (*) 2, ALDH2 (*) 2 in humans have not yet been studied.

Finally, it is worth stressing that a large majority of studies on the physiopathology and prognosis of ACM were conducted some years ago, prior to the development of our current understanding regarding the role of genetics in DCM^[67]. According to recent data, a genetic form of DCM could be present in up to 50% of idiopathic DCM cases, and other specific forms of DCM such as peripartum cardiomyopathy have been shown to have a genetic basis in a significant number of cases^[68]. It is therefore possible that patients with ACM could also harbour a genetic substrate that predisposes them to this form of cardiomyopathy.

Further research is required to determine the definitive role of genetics on ACM pathophysiology.

NATURAL HISTORY OF ALCOHOLIC CARDIOMYOPATHY

In spite of the high prevalence of excessive alcohol consumption and of its consideration as one of the main causes of DCM, only a small number of studies have analysed the long-term natural history of ACM. Unfortunately, all the available reports were completed at a time when a majority of the current heart failure therapies were not available (Table 1).

Furthermore, there are conflicting data among studies regarding the prognosis of the condition, with some

Table 1 Key studies on the long-term prognosis of alcoholic cardiomyopathy

Ref.	Definition alcohol intake/cardiopathy criteria	Number of patients	NYHA III-IV	% Abstinent	Follow-up	Mortality or heart transplant
McDonald <i>et al</i> ^[69]	> 6 beers/d or 2 quarts of wine/wk or 1 fifth of whisky/wk (44 patients consumed > 6 yr) Cardiomegaly with HF signs or symptoms	48	N/A	N/A	N/A	40%
Demakis <i>et al</i> ^[70]	> 8 oz or 1 L wine or 2 L of beer (ca. 90 g) ≥ 5 yr < 50 years old, HF and cardiothoracic ratio > 0.5	57	100%	31%	40.5 mo	57% in persistent drinkers 24% in non-drinkers
Haissaguerre <i>et al</i> ^[9]	> 80 g/d; ≥ 5 yr LVEF < 55% or LVEF 55%-59% and LVEDV 115 mL/m ²	110	N/A	44%	38.8 mo	50% in persistent drinkers 6% in non-drinkers
Prazak <i>et al</i> ^[12]	> 80 g/d; ≥ 5 yr Heart failure and LVEF < 50%	23	52%	N/A	N/A	19% (10-yr survival)
Fauchier <i>et al</i> ^[11]	> 80 g/d; ≥ 5 yr DCM: WHO definition and hospitalization or arrhythmia	50	44%	45%	47 ± 40 mo	50% non-drinkers 70% in persistent drinkers
Gavazzi <i>et al</i> ^[10]	> 80 g/d; ≥ 5 yr or 100 mg/d 2 yr LVEF < 50 and HF or arrhythmia	79	35%	74%	59 ± 35 mo	Overall: 59% 55% in non-drinkers, 73% in persistent drinkers

HF: Heart failure; LVEF: Left ventricular ejection fraction; LVEDV: Left ventricular end-diastolic volume; DCM: Dilated cardiomyopathy; WHO: World Health Organization.

showing overall mortality near 60% and others showing a mortality rate of only 19% (Table 1).

The first paper to assess the natural history and long-term prognosis of ACM was published by McDonald *et al*^[69] in 1971. He recruited 48 patients admitted to hospital with cardiomegaly without a clear aetiology and severe alcoholism. Patients were treated with diuretics, digitalis and vitamin B. During the follow-up, which varied significantly, 19 patients died (40%). The only factor to predict a poor outcome was the duration of symptoms before admission.

Demakis in 1974 recruited 57 patients with ACM^[70]. The patients were drinkers of an amount of alcohol equivalent to > 90-100 g of alcohol per day for at least 5 years. During an average follow-up period of 40.5 mo, 24 deaths occurred among the 57 patients (42%). The adverse prognostic factors found in this study were lasting severe alcohol intake and the duration of HF symptoms.

In 1996, Prazak compared the evolution of a cohort of 42 individuals with idiopathic DCM and that of another group of 23 patients diagnosed with ACM who were seen between the years 1981 and 1992^[12]. The populations were homogeneous and showed no clinical or haemodynamic differences at the beginning of the study. After 10 years of follow-up, the authors concluded that patients with ACM had better prognosis than patients with idiopathic DCM. Survival rates after 1, 5 and 10 years were 100%, 81% and 81%, respectively, in the ACM group, and 89%, 48% and 30% among those with idiopathic DCM. The predictive factors of poor prognosis that were identified were of a clinical nature: New York Heart Association (NYHA) functional class III-IV, presence of hepatojugular reflux and congestion. The left ventricular volumes, ejection fraction and filling pressures were only predictors of prognosis among patients with idiopathic DCM.

The latest two papers to be published, unlike previ-

ous papers, reported worse outcomes for ACM patients compared to DCM patients. In the first of these studies, Fauchier *et al*^[11] studied 50 patients with ACM and 84 patients with DCM between 1986 and 1997. Although up to 81% of ACM patients received an ACEI, none received beta-blockers and the use of spironolactone was not specified, although it was probably quite low. Also, current common cardiac therapies such as ICD and CRT devices were not used because of the period when the study was conducted. After a follow-up period of 47 mo, a significantly higher survival rate was observed among patients with DCM compared to patients with ACM. In this study, the only independent predictor of cardiac death was alcohol abstinence.

In the second study, Gavazzi led a multicentre study in which, from 1986 to 1995, 79 patients with ACM and 259 patients with DCM were recruited^[10]. The average duration of follow-up was 59 ± 35 mo. Transplant-free survival after 7 years was worse among patients with ACM than among those with DCM (41% *vs* 53%). Among patients who continued drinking heavily, transplant-free survival was significantly worse than in non-drinkers (27% *vs* 45%). No other predictors were described.

Considering all the works conducted to date, it is clear that new studies on the natural history of ACM are needed, including patients treated with contemporary heart failure therapies. In light of the available data, new studies will help to clarify the current prognosis of ACM compared to DCM and to determine prognostic factors in ACM that might differ from known prognostic factors in DCM.

TREATMENT

To date, none of the ACM studies have proposed a treatment for ACM other than that recommended for DCM in current HF guidelines.

From the data provided in the available ACM studies, it appears that patients who received an ACEI globally showed improved prognosis. In contrast, beta-blockers, similar to aldosterone inhibitors, however beneficial they may be, have thus far not yielded sufficient data on their efficacy in relation to this disease.

Regarding ICD and CRT implantation, the same criteria as in DCM are used in ACM, although it is known that excessive alcohol intake is specifically linked to ventricular arrhythmia and sudden cardiac death^[71]. As it is not uncommon in ACM for patients to experience a significant recovery of systolic function, it is particularly challenging in this disease to decide the most appropriate time to implant an ICD and whether it is necessary to replace a previously implanted device. Future studies in ACM should also address this topic, which has important economic consequences.

EFFECTS OF ALCOHOL WITHDRAWAL

Complete alcohol withdrawal is usually recommended to all patients with ACM. For tens of years, the literature has documented many clinical cases or small series of patients who have undergone a full recovery of ejection fraction and a good clinical evolution after a period of complete alcoholic abstinence. The need for complete withdrawal, however, is still disputed.

Demakis *et al*^[70] in 1974 divided a cohort of 57 ACM patients according to the evolution of their symptoms during follow-up. The sub-group of patients in whom symptoms improved was made up of a larger proportion of non-drinkers (73%), compared to 25% in the group who did not improve, or 17% in the group whose condition worsened. However, a possible confusion factor was identified because the group with clinical improvement also exhibited a shorter evolution of the symptoms and the disease.

Guillo *et al*^[17] in 1997 described the evolution of 9 ACM patients who had been admitted. He divided this cohort into two groups according to the evolution of the ejection fraction during 36 mo in which no deaths were recorded. The 6 subjects who experienced a clear improvement in their ejection fraction had fully refrained from drinking. Conversely, the 3 subjects recording a less satisfactory evolution had persisted in their consumption of alcohol. It should be noted that a moderate drinker included in this latter group showed an improvement of his ejection fraction.

The natural history and long-term prognosis studies of Gavazzi *et al*^[10] and Fauchier *et al*^[11] compared the evolution of ACM patients according to their degree of withdrawal. These authors found a relationship between the reduction or cessation of alcohol consumption and higher survival rates without a heart transplant.

Fauchier *et al*^[11] found that after 47 mo of follow-up, the transplant-free survival of DCM patients was better than that of patients with ACM, but these differences were no longer significant when comparing the DCM

group with the alcoholics who refrained from drinking or significantly reduced their alcohol consumption^[11].

In the study by Gavazzi *et al*^[10], ACM patients who continued drinking exhibited worse transplant-free survival rates after 7 years than those who stopped drinking alcohol (27% *vs* 45%)^[10].

Ballester specifically analysed the effects of alcohol withdrawal on the myocardium using antimyosin antibodies labelled with Indium-111^[72]. This radiotracer has been acknowledged as an indicator of irreversible myocardial damage. Of the 56 patients included in the study, 28 were former drinkers and 28 continued consuming alcohol during the study. Absorption levels of Indium-111 were high in 75% of patients who continued drinking and in only 32% of those who had withdrawn from consuming alcohol.

Of the 19 patients who were studied 9 ± 4 mo after withdrawal, the average absorption level decreased from an average HLR of 1.76 ± 0.17 to 1.55 ± 0.19 and this was associated with a significant improvement in the ejection fraction, from $30\% \pm 12\%$ to $43\% \pm 16\%$ ($P < 0.01$).

Data supporting the beneficial effect of continuing with alcohol intake at moderate levels in ACM patients arose from the observation that published studies evaluating the effect of alcohol abstinence included ACM patients who reduced their alcohol intake to low/moderate levels alongside ACM patients who stopped their alcohol intake altogether^[9-12]. Also, low to moderate daily alcohol intake was proved to be a predictor of better prognosis for both ischemic cardiomyopathy and heart failure regardless of the presence of coronary disease^[12].

Additionally, echocardiographic data suggest that subjects who do not fully withdraw from alcohol consumption, but who reduce it to moderate amounts recover LVEF in a similar manner to strict non-drinkers. Thus, Nicolás *et al*^[73] studied the evolution of the ejection fraction in 55 patients with ACM according to their degree of withdrawal. The population was divided into 3 groups according to their intake volume during the follow-up period. At the end of the first year, no differences were found among the non-drinkers, who improved by 13.1%, and among those who reduced consumption to 20-60 g/d (with an average improvement of 12.2%). Conversely, those whose consumption remained in excess of 80 g/d showed an average decline of 3.8% in their ejection fraction.

Thus, although there is a certain degree of consensus regarding the recommendation of full alcohol withdrawal in ACM, it is yet to be resolved whether moderate alcohol consumption is sufficient to achieve an improvement in the prognosis of these patients.

Future studies with a strict classification of non-drinkers and drinkers will help clarify whether complete abstinence is mandatory for ACM patients. In the interim it seems appropriate to continue discouraging any alcohol consumption in these patients, as it would be difficult for them to maintain a limited alcohol intake considering their history of alcohol dependence and abuse.

LIMITATIONS OF ACM STUDIES

In all ACM studies, inclusion of patients is based on patients' self-reported alcohol drinking habits, which may lead to an underestimation of the prevalence of ACM together with problematic identification of patients who abstain and those who continue drinking. Although analytical markers of alcohol consumption, such as average erythrocyte volume and serum gamma-glutamyltranspeptidase levels, could be an aid to establish abstinence or persistence of alcohol intake in patients, the quantity of alcohol intake is dependent on the patients' report. Furthermore, in many of these reports, comorbid conditions, especially myocarditis and other addictions such as cocaine and nicotine, were not reported.

As pointed out before, the current accepted definition of ACM probably underestimates the number of women affected by the disease. Alcohol affects heart function and is dependent on the quantity of alcohol that the heart is exposed to. Women typically have a lower BMI than men, and therefore the same alcohol exposure can be achieved with lower alcohol intake.

CONCLUSION

ACM is an important clinical entity known since the 19th century. Epidemiological and experimental studies link excessive alcohol intake to the development of DCM. Although not based on solid experimental or epidemiological data, the currently accepted definition of ACM requires chronic exposure to > 80 g/d of alcohol for > 5 years. There is a surprising paucity of clinical data on ACM prognosis and particularly on ACM evolution under modern HF therapies. In the absence of robust data, current therapy of ACM should include complete alcohol abstinence along with all the therapies recommended to treat DCM. Further studies in the field of ACM are required.

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Interferon- γ and other inflammatory mediators in cardiomyocyte signaling during Chagas disease cardiomyopathy

Ludmila Rodrigues Pinto Ferreira, Amanda Farage Frade, Monique Andrade Baron, Isabela Cunha Navarro, Jorge Kalil, Christophe Chevillard, Edecio Cunha-Neto

Ludmila Rodrigues Pinto Ferreira, Amanda Farage Frade, Monique Andrade Baron, Isabela Cunha Navarro, Jorge Kalil, Edecio Cunha-Neto, Laboratory of Immunology, Heart Institute (InCor), School of Medicine, University of São Paulo, 05403-001 São Paulo, Brazil

Ludmila Rodrigues Pinto Ferreira, Amanda Farage Frade, Monique Andrade Baron, Isabela Cunha Navarro, Jorge Kalil, Edecio Cunha-Neto, Division of Clinical Immunology and Allergy, School of Medicine, University of São Paulo, 05403-001 São Paulo, Brazil

Ludmila Rodrigues Pinto Ferreira, Amanda Farage Frade, Monique Andrade Baron, Isabela Cunha Navarro, Jorge Kalil, Edecio Cunha-Neto, Institute for Investigation in Immunology (iii), INCT, 05403-001 São Paulo, Brazil

Christophe Chevillard, Aix-Marseille Université, INSERM, GIMP UMR_S906, 13385 Marseille, France

Author contributions: Ferreira LRP prepared the multiple drafts and wrote the manuscript; Chevillard C and Cunha-Neto E prepared the framework of the article; Frade AF, Baron MA and Navarro IC helped with scientific discussions; Kalil J, Chevillard C and Cunha-Neto E improved the quality of the draft.

Correspondence to: Edecio Cunha-Neto, MD, PhD, Researcher, Associate Professor of Medicine, Laboratory of Immunology, Heart Institute (InCor), School of Medicine, University of São Paulo. Av. Dr. Eneas de Carvalho Aguiar, 44 BL 2, 9^o andar, 05403-001 São Paulo, Brazil. edecunha@usp.br

Telephone: +55-11-26615906 Fax: +55- 11-26615953

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carditis with increased production of interferon (IFN)- γ , produced by the CCC myocardial infiltrate and detected at high levels in the periphery. IFN- γ has a central role in the cardiomyocyte signaling during both acute and chronic phases of *T.cruzi* infection. In this review, we have chosen to focus in its pleiotropic mode of action during CCC, which may ultimately be the strongest driver towards pathological remodeling and heart failure. We describe here the antiparasitic protective and pathogenic dual role of IFN- γ in Chagas disease.

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Key words: Chagas disease; *Trypanosoma cruzi*; Interferon-gamma; Gene expression; Cardiomyopathy

Core tip: Chagas disease cardiomyopathy (CCC) occurs in 30% of those infected with the protozoan *Trypanosoma cruzi*, endemic in Latin America. It is an inflammatory cardiomyopathy with a worse prognosis than cardiomyopathies of other etiologies. Interferon (IFN)- γ is the main cytokine produced locally and induces strong signaling in cardiomyocytes. This review focuses on the pleiotropic protective and pathogenic effects of IFN- γ on CCC.

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Abstract

Chagas disease cardiomyopathy (CCC), the main consequence of *Trypanosoma cruzi* (*T.cruzi*) infection, is an inflammatory cardiomyopathy that develops in up to 30% of infected individuals. The heart inflammation in CCC patients is characterized by a Th1 T cell-rich myo-

INTRODUCTION

Chagas disease cardiomyopathy (CCC) is a particularly

aggressive inflammatory dilated cardiomyopathy that occurs decades after the initial infection with the obligate intracellular parasite *Trypanosoma cruzi* (*T. cruzi*) in 30% of infected individuals^[1]. *T. cruzi* infection affects 10 million subjects in endemic areas of South and Central America and migratory waves have taken patients to the United States, Europe and Japan^[2-4]. Patients with CCC have a worse clinical progression and survival than those with cardiomyopathy of other etiologies. The development of CCC is associated with inflammation and activation of the immune system, with a local increased cardiac production of cytokines by the heart-infiltrating T cells and other mononuclear cells^[5]. These mononuclear cells infiltrating CCC heart tissue express predominantly interferon (IFN)- γ and tumor necrosis factor (TNF)- α , with lower levels of interleukin (IL)-2, IL-4, IL-6 and IL-10. Cytokines like IL-7 and IL-15, which promote T cell survival, are also found to have increased expression in CCC heart tissue^[6,7]. Significant IFN- γ signaling was observed in the myocardium of CCC patients, including genes that are not ordinarily expressed by inflammatory cells^[8]. A similar increase in IFN- γ and TNF- α expression is observed in cardiac tissue from animals infected with *T. cruzi*^[9]. CCC patients have a progressive myocardial remodeling process with hypertrophy and fibrosis causing heart fiber damage, heart conduction abnormalities, arrhythmias, apical aneurysm, heart failure and sudden death^[10,11]. Several hypotheses have been raised to explain the lesions in the myocardium of CCC, which includes persistence of the parasite or its antigens at the inflammatory site and autoimmune tissue damage^[5,12]. There are two drugs available to treat the acute phase of the disease, nifurtimox (nitrofurane) and benznidazole (nitroimidazole). The use of these drugs to treat the acute phase of the disease is widely accepted. However, their use in the treatment of the chronic phase is controversial. There is no specific treatment, against the parasite, that can benefit patients at the chronic stage of Chagas disease^[13]. The undesirable side effects of both drugs are a major drawback in their use, frequently forcing the physician to stop treatment. The treatment of chronic patients consists of control of the symptoms and improvement in quality of life, by preventing cardiovascular complications according to the guidelines for treating heart failure and arrhythmias^[14]. Regardless of the mechanisms underlying the initiation and maintenance of the myocarditis, the bulk of the evidence indicates that the inflammatory infiltrate is a significant effector of heart tissue damage. Our group has demonstrated over the past several years that, aside from direct inflammatory damage, several cytokines and chemokines produced in the myocardium of CCC patients may also have a non-immunological pathogenic effect beyond direct inflammatory tissue damage, *via* modulation of gene and protein expression in cardiomyocytes and other myocardial cell types^[5,7,15,16]. While IFN- γ acts as an immunological mediator during the acute stage of the disease suppressing overt parasitism, in the chronic phase of the disease it will both curtail parasitism and cause tissue damage through immunological and non-

immunological effects entertaining the gradual progression to CCC.

IFN- γ IN HEALTH AND DISEASE

IFN- γ is a protein with 146 amino acid residues, the only member of the type II IFN family, and in humans is encoded by a chromosomal locus separate from type I IFNs, on chromosome 12q24.1 with approximately 5.4 kb and four exons^[17]. IFN- γ is mainly produced by CD4⁺ T helper cell type 1 (Th1) lymphocytes, CD8⁺ cytotoxic lymphocytes, and natural killer (NK) cells, but can also be produced by other cells, such as B cells, NKT cells, and professional antigen-presenting cells (APCs). Cytokines secreted by APCs, most notably IL-12 and IL-18, control IFN- γ production and differentiation of cells capable of producing the cytokine. Interaction of macrophages and other APCs with pathogen-associated molecular patterns (PAMPs) induces secretion of IL-12 and chemokines. These chemokines attract inflammatory cells to the site of inflammation, and IL-12 promotes IFN- γ synthesis in these cells^[18]. Negative regulators of IFN- γ production include IL-4, IL-10, transforming growth factor (TGF)- β , and glucocorticoids^[19]. Animal models as well the analysis of different human diseases are good examples of the paradoxical roles of IFN- γ . Mice lacking IFN- γ and its receptor (IFNGR) showed no developmental defects, and their immune system appeared to develop normally^[20]. However, these mice show deficiencies in natural resistance to infection. In humans, inactivating mutations of the human IFNGR1 or IFNGR2 chains show clinical presentation similar to the mouse models. At the same time IFN- γ can be beneficial in infectious diseases where it strengthens cellular defense mechanisms and favors the generation of specific immunity, and can be disease-promoting as described in non-infectious diseases. Reifenberg *et al*^[21] have shown that SAP-IFN- γ transgenic mice, which constitutively express IFN- γ in their livers, developed chronic active myocarditis. These mice exhibited IFN- γ -mediated cardiotoxicity with left ventricular dilation and impaired systolic function, a true cardiomyopathy^[21]. Morino *et al*^[22] have reported a case of cardiomyopathy in a renal cell carcinoma patient treated with IFN- γ . In humans, IFN- γ is also implicated in the pathology of diseases such as systemic lupus erythematosus^[23], insulin-dependent diabetes mellitus^[24] and multiple sclerosis^[25]. Like other cytokines, the IFN- γ coding region is invariant with no reported polymorphisms. However, single nucleotide polymorphisms (SNPs) in intronic regions have been described and a microsatellite polymorphism consisting of a dinucleotide (CA) repeat in the first intron is the one most extensively studied as it is correlated with high IFN- γ production^[26]. An association between IFN- γ SNPs and diseases like rheumatoid arthritis has been reported^[27,28]. Nevertheless, as a cytokine with ambiguous effects, IFN- γ polymorphisms are correlated with increased longevity^[25]. It has been proposed that a slightly dampened inflammatory status caused by an IFN- γ polymorphism, while not enough to significantly impact on

the individual's ability to clear infection, may prevent or defer inflammation-related diseases such as cardiovascular disease, neurodegeneration, osteoarthritis, osteoporosis, and diabetes^[29]. In experimental *T. cruzi* infection, it has been shown by several investigators that IFN- γ can enhance macrophage killing of the parasite *in vitro* and increase resistance to an infectious challenge *in vivo*, an effect dependent on the *de novo* synthesis of TNF- α and NO by infected macrophages^[9,30,31]. It has also been demonstrated that parasite-induced IFN- γ produced during *T. cruzi* infection by T and NK cells is involved in resistance to infection and protection in mice. This protection seems to be dependent on the IFN- γ /TNF- α pathway^[31].

IFN- γ INDUCED SIGNALING IN CARDIOMYOCYTES INFECTED WITH *T. CRUZI*

Although infective *T. cruzi* trypomastigotes are capable of invading a wide variety of tissues and cell types in the vertebrate host, the majority of *T. cruzi* laboratory strains and isolates have tropism for cardiac tissue and/or cardiomyocytes^[32]. The establishment of a long-term infection in the heart and the development of a cardiomyopathy condition are directly related to the ability of *T. cruzi* to infect and persist within cardiomyocytes during the acute phase of infection^[33,34]. Cardiomyocytes are differentiated cells that respond to *T. cruzi* infection by initiating adaptive strategies. These strategies can involve immunological and non-immunological events. For example, during *T. cruzi* infection cardiomyocytes reactivate an embryonic gene expression pattern^[8] (e.g., an increase in expression of atrial natriuretic factor), inhibit apoptosis^[34], increase cell size by producing myofibrils (cardiac myosin heavy chain, several α -actin isoforms, smooth muscle myosin, actin-binding proteins, and collagen) and initiate a hypertrophic program, that are not related to an immunological response to the parasite^[7]. However, these cells are actively integrated in the inflammatory process and can secrete chemokines such as C-C chemokine monocyte chemoattractant protein 1 (JE/MCP-1/CCL2), chemokine (C-C motif) ligand 5 (RANTES/CCL5), keratinocyte chemoattractant (KC/CXCL3), macrophage inflammatory protein (MIP-2/CXCL2), Mig/CXCL9, and cytokine-responsive gene-2 (Crg-2/CXCL10), and the cytokines TNF- α , IL-1 β and inducible NO synthase (iNOS)^[35]. These chemokines will drive the early influx of leukocytes, and influence T-helper cell recruitment and local IFN- γ production defining the inflammatory infiltrate in the hearts during experimental *T. cruzi* infection and, presumably, also in acutely infected patients. It was recently demonstrated that there is a segregation of CD8⁺ cell populations in the heart in *T. cruzi* infected mice into two groups: CD8⁺ T cells producing perforin and no IFN- γ (IFN- γ ^{neg} Pfn⁺) and perforin-negative and IFN- γ -producing cells (IFN- γ ⁺ Pfn^{neg}). These data supported the idea that CD8⁺ Pfn⁺ T-cells are involved in cardiomyocyte injury during *T. cruzi* infection, whereas CD8⁺ IFN- γ ⁺

cells play a beneficial role in cardiomyocyte damage^[36].

IFN- γ A DUAL ROLE IN CHAGAS DISEASE

A dual role in pathogenesis and protection during Chagas disease was described for IFN- γ and other cytokines, such as TNF- α ^[37]. Bahia-Oliveira *et al.*^[38], taking into account only the inflammatory actions of the cytokine, also described the dual role of IFN- γ during chronic Chagas disease. Our observations from the standpoint of the pleiotropic biological effects, both inflammatory and non-inflammatory, in Chagas disease made us remodel the concept as will follow in these review. During *T. cruzi* infection, once the inflammatory process starts, IFN- γ will be produced by Th1 cells and act as a prime inflammatory cytokine in different pathways of the immune system, such as upregulating MHC class I and class II molecules, suppressing Th2 immune responses by antagonism of IL-4 production, inducing high levels of antigen presentation and activating macrophages^[18]. Our group has demonstrated the importance of IFN- γ , TNF- α and several chemokines in CCC by showing that they play a role in the generation of the inflammatory infiltrate^[8,15,39]. CCC patients have an increased peripheral production of IFN- γ and TNF- α when compared to patients with the asymptomatic/indeterminate form. On the other hand, IFN- γ has direct effects on cardiomyocytes and perhaps other cells of the myocardium^[8]. In the following sections we describe in detail the dual mechanism of IFN- γ during Chagas disease (acute and chronic phases) as illustrated in Figure 1.

IFN- γ ACTS AS AN IMMUNOLOGICAL MEDIATOR INDUCING PROTECTION DURING THE ACUTE PHASE AND ALLOWING CONTROL OF CHRONIC PARASITISM

Data from animal models and from the earliest stages in a proportion of naturally infected individuals has shown that inflammatory cytokines such as IFN- γ play a central role in acute *T. cruzi* infection. During invasion, *T. cruzi* or its derived molecules like DNA and glycosylphosphatidylinositol-anchored mucin-like glycoproteins derived from trypomastigotes forms (tGPI-mucins) can stimulate the host cutaneous cells, macrophages, cardiomyocytes and dendritic cells (as seen in *in vivo* and *in vitro* infection) to produce mediators that will trigger a local inflammatory response^[40]. This activation will induce these cells to promptly release pro-inflammatory cytokines such as IL-1, IL-6, IL-12, IL-18, IL-27 and TNF- α and further activate other inflammatory cells. These cytokines will participate in the control of the infection, killing the parasite with the help of NO production *via* iNOS/NOS2. *T. cruzi*-specific T cells will produce IFN- γ , which in conjunction with macrophages producing TNF- α will migrate with other blood leukocytes to the site of inflam-

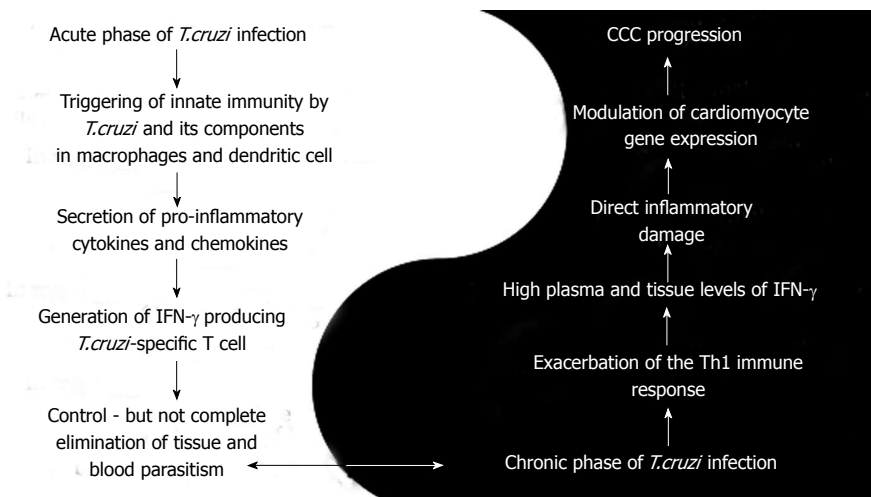


Figure 1 Interferon- γ a dual role in Chagas disease (acute and chronic phases). CCC: Cardiomyopathy; INF: Interferon.

mation in response to chemokines such as CCL2, CCL3, CCL4, CCL5, CXCL10 and CCR5^[41]. The blockade of one, CCR5, by Met-RANTES significantly decreased the intensity of cardiac inflammatory infiltrate, suggesting that lymphocyte migration to the myocardium during acute infection is dependent on CCR5 ligands^[42,43]. IFN- γ -inducible adhesion molecules, such as fibronectin and VCAM-1, can also be detected at high levels in cardiac tissue from *T. cruzi*-infected mice^[44]. Few studies have investigated the immunology of the acute phase infection in human patients. It has been described that acutely infected children display increased expression of inflammatory cytokines, such as circulating IL-6 and TNF- α ^[45] and increased production of IFN- γ by mononuclear cells^[46]. Serum C-reactive protein (CRP) and IL-6 concentrations have also been shown to increase in children infected with *T. cruzi* during the acute phase, but not in the chronic phase of Chagas disease^[47].

IFN- γ INDUCES DISEASE PROGRESSION DURING THE CHRONIC PHASE ACTING AS A NON-IMMUNOLOGICAL MEDIATOR OF TISSUE DAMAGE

During the chronic phase of *T. cruzi* infection, CCC patients have an exacerbation of the Th1 immune response compared with those with the indeterminate form of Chagas disease. It was observed that CCC patients displayed greater cytokine production (Table 1) by mononuclear cells, higher plasma levels of TNF- α and IFN- γ and an increased number of IFN- γ -producing CCR5⁺CXCR3⁺CD4⁺ and CD8⁺ T cells, with reduced numbers of IL-10-producing and FoxP3⁺ regulatory T cells^[15,48-50]. It has been hypothesized that this increased production of IL-10 by regulatory T cells restricts Th1 T cell differentiation and IFN- γ production in the majority of chronically *T. cruzi*-infected individuals, leading to the asymptomatic form of the disease^[15,48-50]. Aside from the delayed hypersensitivity type of tissue damage classically seen in tissue lesions induced by IFN- γ , with cardiomyo-

cyte loss and fibrosis, the local production of IFN- γ in CCC heart lesions can induce profound changes in the cardiomyocyte gene expression pattern as observed by our group using cDNA microarrays. Significant IFN- γ signaling was observed in the myocardium of CCC patients, including genes that are not ordinarily expressed by inflammatory cells. We have observed that 15% of the genes selectively upregulated in CCC are IFN- γ -inducible genes, including inflammatory response genes expressed by the infiltrating inflammatory cells (*e.g.*, cytokine receptors, immunoglobulin, T cell receptor genes). Several IFN- γ modulated genes are not expressed by inflammatory cells, including angiotensin II receptor 2, fatty acid-binding protein 5, cardiovascular 27-kd Hsp and genes encoding a number of proteins involved in oxidative phosphorylation and lipid catabolism in the CCC myocardium, compared with idiopathic dilated cardiomyopathy or donor myocardium^[8]. cDNA microarray experiments in mice infected with *T. cruzi* showed changes in oxidative phosphorylation and depressed energy metabolism^[51] and respiratory chain complexes with a reduced ATP-generating capacity^[52]. Moreover, expression profiling in hearts of mice infected by *T. cruzi* also showed diminished myocardial energy metabolism and altered oxidative phosphorylation^[51,53]. Significantly, mice infected for 100 d showed morphological alterations in the mitochondria and diminished expression of genes from the oxidative phosphorylation pathway, with a detectable reduction in OXPHOS-mediated mitochondrial ATP production^[51]. Thus, both IFN- γ and *T. cruzi* infection can depress energy metabolism to reduce myocardial ATP generation, which has potential consequences for myocardial contractility, electric conduction and rhythm. Interestingly, one of the genes downregulated in CCC hearts, SERCA Ca²⁺-ATPase is repressible by IFN- γ and is also involved in cardiac metabolism. *In vitro* experiments have shown that IFN- γ may induce profound changes in the cardiomyocyte gene expression program, including induction of atrial natriuretic factor and of the hypertrophic gene expression program, which can ultimately lead to heart dilation and heart failure^[8]. Other inflammatory mediators and chemokines such as IL-18 and CCR7 ligands, up-

Table 1 Cytokine and chemokine expression in Chagas disease and animal models^[80]

Cytokines/ chemokines	Phase (acute /chronic/IND /severe/ moderate CCC)	Host (mouse/ human)	Organ/cell type	Ref.
IFN- γ	Severe CCC	Human	Mononuclear cells	[15,49]
IFN- γ	Severe CCC	Human	Myocardium	[63,64]
IFN- γ	Severe CCC	Human	Heart-infiltrating T cells	[15]
IFN- γ	IND, Severe CCC	Human	Plasma	[15,65,66]
TNF- α	Severe CCC	Human	Mononuclear cells	[15,49]
TNF- α	Severe CCC	Human	Heart-infiltrating T cells	[15]
TNF- α	Severe CCC	Human	Myocardium	[63,64]
TNF- α	IND and Severe CCC	Human	plasma	[15,65,66]
IFN- γ	Acute/ chronic	Mouse	Heart	[67-69]
TNF- α	Acute/ chronic	Mouse	Heart	[70]
IL-6	Severe CCC	Human	Heart-infiltrating T cells	[15,63,64]
IL-2	Severe CCC	Human	Heart-infiltrating T cells	[15,63,64]
IL-4	Severe CCC	Human	Heart-infiltrating T cells	[15,63,64]
IL-10	Severe CCC	Human	Heart-infiltrating T cells	[15, 63, 64]
IL-7	Severe CCC	Human	Myocardium	[71]
IL-15	Severe CCC	Human	Myocardium	[71]
IL-12	Acute	Mouse	Mononuclear cells	[72]
IL-18	Acute	Mouse	Mononuclear cells	[73]
IL-10	Acute	Mouse	Mononuclear cells	[74-76]
TGF- β	Acute	Mouse	Mononuclear cells	[74-76]
IL-17	Chronic	Mouse	Mononuclear cells	[77]
CCL2, CXCL10, CXCL9 (mRNA)	Severe CCC	Human	Myocardium	[8]
CCR2, CXCR3 (mRNA)	Severe CCC	Human	Myocardium	[8]
CCR5, CXCR3	Severe CCC, IND	Human	Mononuclear cells	[48]
CCL5, CXCL9, CXCL10	Chronic	Mouse	Cardiomyocytes	[35]
CCR5	Chronic	Mouse	Heart	[43,78]
CCL5, CCL4, CXCR3 (mRNA)	Chronic	Dog	Heart	[79]

CCC: Cardiomyopathy; INF: Interferon; IL: Interleukin; TNF: Tumor necrosis factor.

regulated in the CCC myocardium^[39], induce cardiomyocyte hypertrophy and molecules involved in the fibrotic process^[54-56]. Transgenic mice overexpressing CCL2,

TNF- α or IFN- γ in the myocardium develop myocardial hypertrophy and ventricular dilation^[21,57,58]. Inflammatory cytokines may also affect myocardial energy metabolism, and ventricular dysfunction is associated with reduced energy metabolism^[59,60]. Treatment of cardiomyocytes with IFN- γ inhibited oxidative metabolism and ATP production^[61] and reduced gene and protein expression of creatine kinase, which is responsible for translocation of mitochondrial ATP to the sarcoplasm in cultured human skeletal muscle cells^[62]. We observed that the myocardium of CCC patients displays reduced expression of some key energy metabolism enzymes, including isoforms of creatine kinases, Krebs cycle enzymes, and members of the ATP synthase complex, in comparison with the myocardium of patients with non-inflammatory cardiomyopathies and heart donors (unpublished observations), which could be partly due to IFN- γ inflammatory cytokine signaling. cDNA Microarray experiments in mice experimentally infected with *T. cruzi* showed changes in oxidative phosphorylation and depressed energy metabolism^[51], and respiratory chain complexes with a reduced ATP-generating capacity^[52]. Thus, both IFN- γ and *T. cruzi* infection can depress energy metabolism, reducing myocardial ATP generation, with potential consequences for myocardial contractility, electrical conduction and rhythm. Taken together, data show that, apart from the direct inflammatory damage, the non-immunological effects of IFN- γ in the myocardium may play a significant pathogenic role in CCC, resulting in disease progression observed by a high degree of heart failure-inducing hypertrophy and fibrosis. The in-depth understanding of these pathways may lead to the development of new therapies for CCC.

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WJC 6th Anniversary Special Issues (4): Congestive heart failure

Innate immune receptors in heart failure: Side effect or potential therapeutic target?

Katharina B Wagner, Stephan B Felix, Alexander Riad

Katharina B Wagner, Stephan B Felix, Alexander Riad, Department of Cardiology and Internal Medicine B, University Medicine Greifswald, Sauerbruchstrasse, 17475 Greifswald, Germany

Stephan B Felix, Alexander Riad, DZHK (German Centre for Cardiovascular Research), Partner site Greifswald, Sauerbruchstrasse, 17475 Greifswald, Germany

Author contributions: Wagner KB wrote the manuscript, literature research; Felix SB and Riad A edited and wrote the manuscript.

Correspondence to: Alexander Riad, MD, Department of Cardiology and Internal Medicine B, University Medicine Greifswald, Sauerbruchstrasse, 17475 Greifswald, Germany. riad@uni-greifswald.de

Telephone: +49-3834-8680500 Fax: +49-3834-8680502

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Abstract

Heart failure (HF) is a leading cause of mortality and morbidity in western countries and occasions major expenses for public health systems. Although optimal medical treatment is widely available according to current guidelines, the prognosis of patients with HF is still poor. Despite the etiology of the disease, increased systemic or cardiac activation of the innate immune system is well documented in several types of HF. In some cases there is evidence of an association between innate immune activation and clinical outcome of patients with this disease. However, the few large trials conducted with the use of anti-inflammatory medication in HF have not revealed its benefits. Thus, greater understanding of the relationship between alteration in the immune system and development and progression of HF is urgently necessary: prior to designing therapeutic interventions that target pathological inflammatory processes in preventing harmful cardiac effects of immune modulatory therapy. In this regard, relatively

recently discovered receptors of the innate immune system, *i.e.*, namely toll-like receptors (TLRs) and nod-like receptors (NLRs)-are the focus of intense cardiovascular research. These receptors are main up-stream regulators of cytokine activation. This review will focus on current knowledge of the role of TLRs and NLRs, as well as on downstream cytokine activation, and will discuss potential therapeutic implications.

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Key words: Heart failure; Innate immune system; Toll-like receptors; Inflammation

Core tip: Heart failure (HF) is a leading cause of morbidity and mortality despite of current medical and interventional treatment. Activation of the innate immune system leading to or contribute to advanced HF is focus of intense and growing research. This review will focus on the role of innate immune receptors in HF. We will discuss the current knowledge about the correlation of innate immune activation and the clinical course in HF. In addition, we will comment on potential therapeutic implications of modulating the immune system in this syndrome.

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INTRODUCTION

Heart failure (HF) is a one of the leading cause of mortality and morbidity. In developed countries, 1% to 2% of the adult population suffers from this syndrome^[1]. In

patients ≥ 70 years of age, the prevalence increases to more than 10%^[2]. Although approximately 50% of HF patients have preserved left ventricular (LV) ejection fraction^[1], this review will focus on systolic HF, owing to the lack of data on the influence of the immune system on HF with preserved LV ejection fraction.

The etiology of HF is manifold. Systolic HF arises in more than 60% of cases from coronary artery disease (CAD). Among others, dilated cardiomyopathy, myocarditis, alcohol abuse, and chemotherapy are relevant and often reasons for HF. Current treatment of systolic HF has been documented in a large number of randomized, controlled clinical trials^[1]. These studies clearly demonstrate the benefits of drugs such as β -blockers, angiotensin, converting enzyme inhibitors, angiotensin receptor antagonists, mineralocorticoid receptor blockers, and new drugs such as ivabradine. These agents reduce mortality and/or improve clinical symptoms of chronic systolic HF by suppression of the renin-angiotensin, aldosterone system, neurohumoral activation and ion channels. In addition to medical treatment, mechanical interventions such as resynchronization therapy have also proven beneficial in selected patients^[3]. However, despite current optimal HF treatment, the prognosis of these patients is still poor and is comparable to neoplastic diseases. This underscores the need for additional therapeutic options. Many different pathophysiological and therapeutic concepts are at the focus of intense current research. Despite various etiologies, there is a growing body of evidence in this context from more than two decades of research for innate immune activation-systemic and/or local-in a significant number of patients and in experimental studies^[4]. The innate immune system represents the first line of host defense against pathogens. This system is composed of diverse cellular components including granulocytes (basophils, eosinophils and neutrophils), mast cells, monocytes/macrophages, dendritic cells, and natural killer cells^[5]. These cells respond to noxious stimuli and conditions, including infections and tissue injuries that can trigger inflammatory responses^[6]. Pro-inflammatory cytokines, which can be excessively produced by immune cells, have been identified over the last decades as “downstream effectors” of the innate immune system^[7]. Moreover, several clinical studies that apply pharmacological cytokine inhibition have been carried out for various diseases^[4]. However, in HF, suppression of the cytokine tumor necrosis factor (TNF) alpha has failed to show a benefit in patients^[8]. One reason for this failure may be a general underestimation of the complexity of the innate immune system. The regulation of cytokines is indeed not well understood^[7]. In this regard, the discovery of so-called pattern recognition receptors has substantially enlarged understanding of the innate immune system. Two families of receptors, *i.e.*, toll-like receptors (TLRs) and nod-like receptors (NLRs)-have been relatively recently discovered; they regulate the innate immune response^[7,9]. This review will discuss the pathophysiology of TLRs and NLRs and their role as therapeutic targets in systolic HF.

TLRS AND NLRs

TLRs

The family of TLRs represents the best known receptor proteins in the innate immune system. The initially discovered TLR4 has by now been known and researched for nearly two decades^[10]. Extensive research has led to discovery of ten functional TLRs in humans, and has enabled detailed decoding of the TLR pathway^[11]. Still, the role of TLRs in autoimmune diseases has not yet been fully understood. All TLR share a cytoplasmic Toll/IL-R homology (TIR) domain^[12]. They reside in different compartments of the cell, with TLR1, 2 and 4-6 on the plasma membrane and TLR3 and 7-10 on intracellular endosomes and lysosomes. In general, cell surface TLRs recognize microbial membrane lipids, and intracellular TLRs respond to microbial nucleic acids^[13]. Furthermore, TLR2 recognizes peptidoglycans, TLR3 dsRNA, TLR4 LPS, TLR7 ssRNA, and TLR9 unmethylated bacterial CpG DNA^[14]. Beneath their role in immune reaction against pathogens, TLRs can also respond to damage-associated molecular pattern molecules (DAMPs). DAMPs include cell, derived particles such as heat shock proteins (HSP) and high mobility group box (HMGB), particles from the extracellular matrix such as fibronectin, and other substances like oxidized low density lipoprotein and free fatty acids^[15]. HSP60 has been shown to activate TLR2 and TLR4 in macrophages^[16]. HSP70 also poses an endogenous stimulus to TLR, which leads to release of nitric oxide and tumor necrosis factor^[17]. In dendritic cells, TLR2 is activated by hyaluronic acid derived from the extracellular matrix^[18]. Upon activation, all TLRs except TLR3 engage the MyD88 pathway. Activated MyD88 forms a complex with IL-1R-associated protein kinases (IRAK4, IRAK1 and IRAK2) (schematic overview see Figure 1). Phosphorylation of IRAK1 leads to activation of tumor necrosis receptor-associated factor (TRAF) 6, which together with IRAK1 forms a new complex. Transforming growth factor-activated kinase (TAK)1, TAK1-binding proteins (TAB)1, TAB2, and TAB3 are recruited to this complex. Upon activation of TAK1 by ubiquitylated TRAF6 IKK- α , IKK- β , and NF- κ B essential modulator (NEMO) form a complex, which degrades I κ B. This leads to translocation of NF- κ B to the nucleus^[19]. NF- κ B regulates transcription of proinflammatory genes, upregulation cell-adhesion molecules and chemokines, and increasing nitric oxide (NO)^[14]. The MyD88-independent pathway is addressed by TLR3 and by TLR4 as an alternative pathway. TLR4 uses the adaptor protein TRIF-related adaptor molecule (TRAM) to activate TIR-domain, containing adapter-inducing interferon- β (TRIF). TRIF can either activate TRAF6-subsequently leading to NF- κ B translocation, or can recruit TRAF3, TBK1, and IKK ϵ . This complex phosphorylates interferon regulatory factor (IRF) 3, which induces its translocation to the nucleus and expression of type I interferone genes^[19]. Several mechanisms aid in the function of TLR signaling, sCD14 has been known

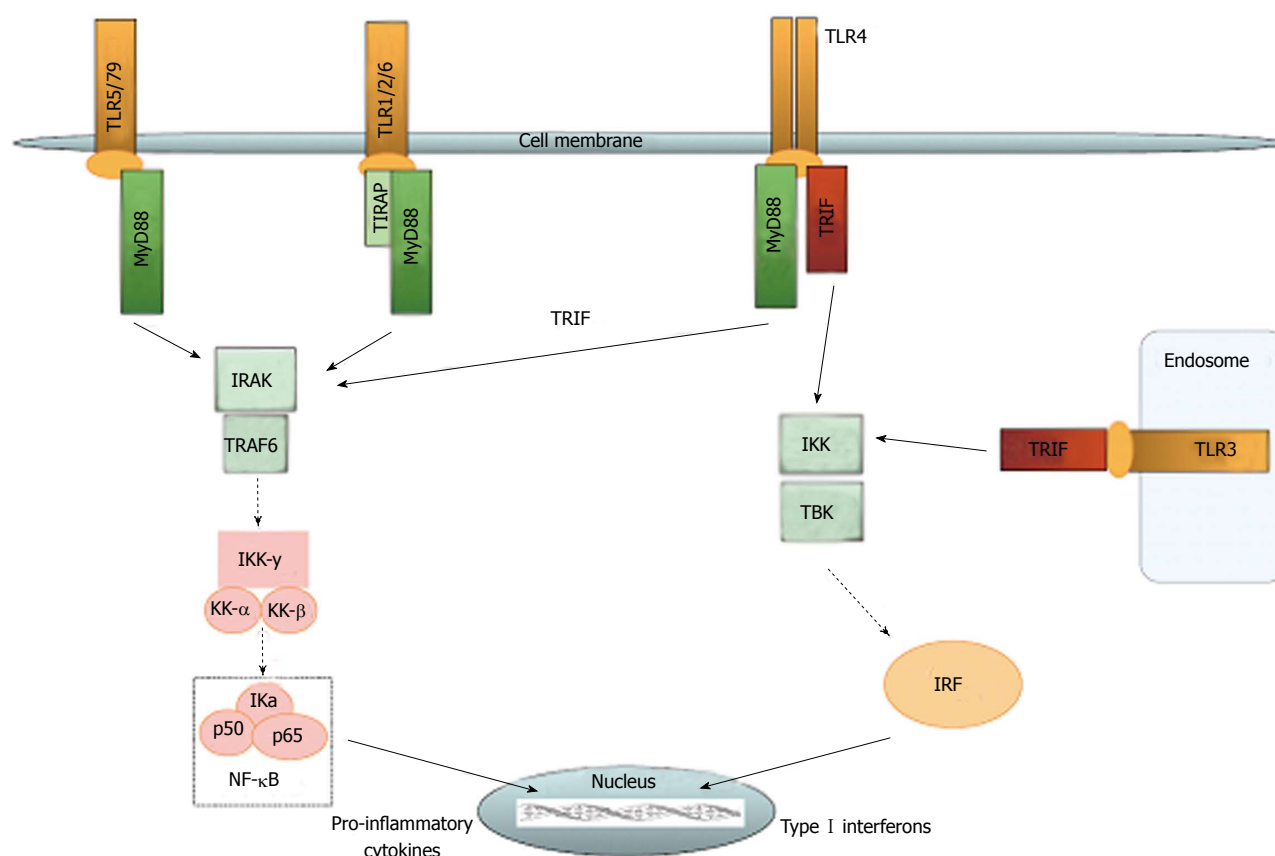


Figure 1 Toll-like receptor signaling. This figure summarizes schematically the complex signalling cascades of the Toll-like receptors. IRF: Interferon regulatory factor; IRAK: Interleukin-1 receptor-associated kinase; TRIF: TIR domain-containing adaptor inducing IFN- β ; TLR: Toll-like receptor; IKK: I κ B kinase; NF- κ B: Nuclear factor κ B; TBK: TANK binding kinase.

to chaperone lipopolysaccharide (LPS) from LPS binding protein to the TLR4/MD2 complex and thus support induction of TNF- α and interleukin-6 (IL-6) production. Recent research has shown that sCD14 is also capable of promoting internalization of TLR4 and activation of the TRIF-dependent pathway^[20]. MHC class II molecules also have the potential of addressing the TLR pathway in a rather unclassical manner. Together with CD40, MHC class II can activate tyrosine kinase Btk, which leads to activation of both the MyD88-and the TRIF-pathways^[21]. Another example for support of the proinflammatory TLR pathway is miR-155. This micro RNA interacts with Src homology 2 domain-containing inositol 5-phosphatase-1 (SHIP-1) and thereby restrains it from its control function^[22]. A potent system such as the TLR proinflammatory pathway requires not only triggering but, perhaps more importantly, control. Recent years have seen establishment of possible control mechanisms for TLR signaling. SHIP-1 is upregulated after LPS stimulation, owing to increased production of transforming growth factor (TGF)- β , and inhibits PI-3 kinase, which consequently blocks TLR-MyD88 and MyD88 independent pathway^[23]. IRAK-M functions as a decoy and prevents IRAK-1 from dissociating MyD88. It suppresses TLR-mediated inflammatory response. IRAK-M knock-out mice demonstrate an increase in inflammatory response and IL-1/TLR-signaling^[24]. IRAK-M can also interfere with TLR2, although

this downregulation is evidently IRAK-1 independent^[25]. Other specific inhibitors are SHP2-which has been shown to inhibit only the TLR3 pathway-and sterile- α and armadillo motif-containing protein (SARM), which blocks only the TRIF-pathway without inhibiting MyD88 signaling^[26,27]. An alternative splice variant of MyD88 is expressed after LPS stimulation. This variant, MyD88s, inhibits phosphorylation of IRAK1 by IRAK4, and leads to a suppression of the TLR pathway^[28]. While microRNA is involved in the promotion of TLR signaling, it also plays an important role in anti-inflammation. miR-146- and miR-21-levels increase after LPS stimulation. miR-146 interacts with TRAF6 and IRAK1, which leads to decreased mRNA levels of both - whereas miR-21 inhibits PDCD4, which is an inhibitor of IL-10^[29]. IKK β , involved in the TLR-pathway, also has anti-inflammatory capacity by virtue of regulating the activation of the prosurvival kinase Akt1^[30]. MHC class I also has a rather untypical function. It can be phosphorylated after TLR activation and can then activate Fps tyrosine kinase, which interferes with TLR signaling^[31]. While evidence suggests a possible pro-inflammatory role of MHC class II, MHC class I evidently supports anti-inflammatory effects.

NLRs

The nucleotide-binding and oligomerization domain

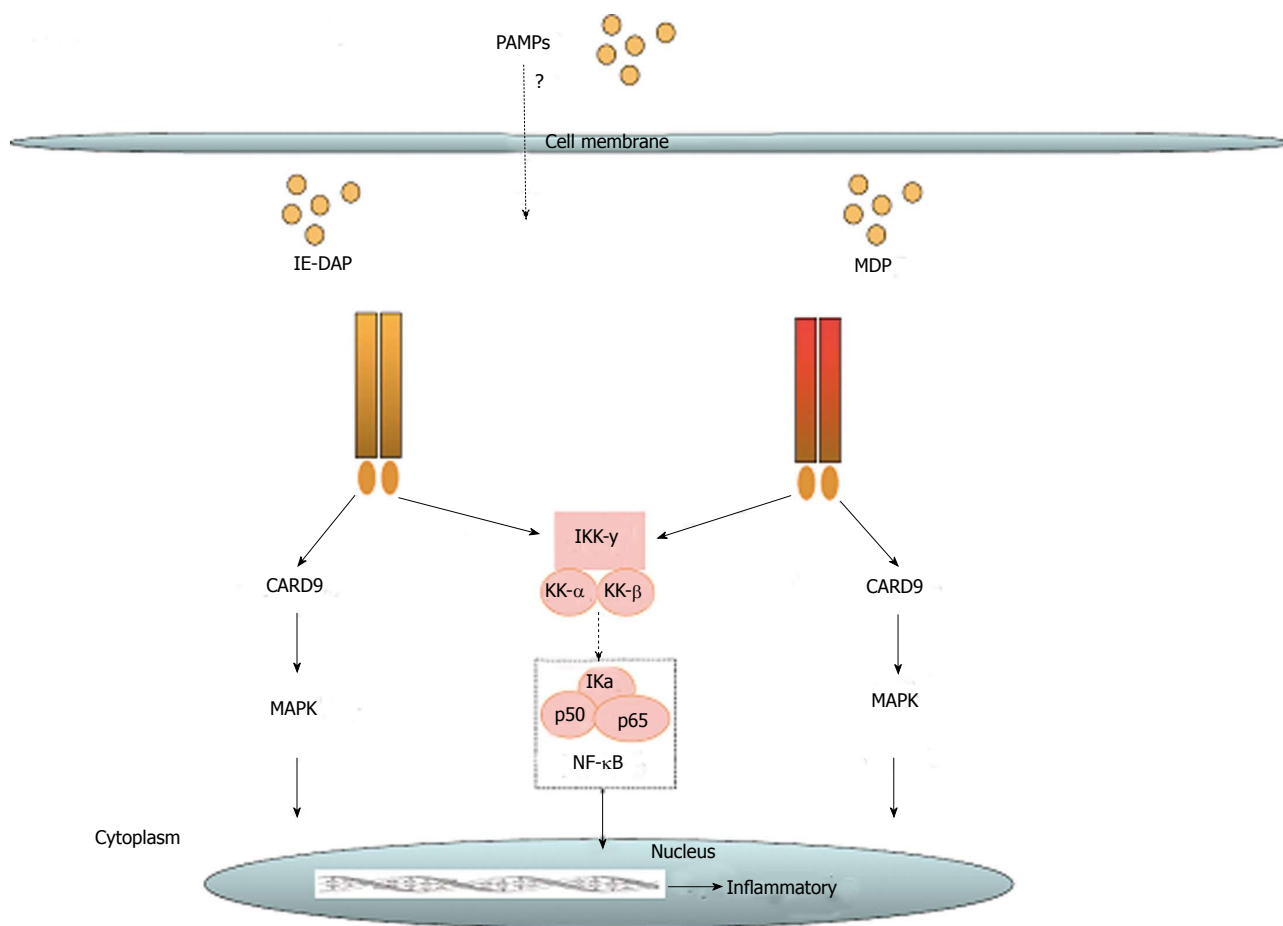


Figure 2 Nod 1 and nod 2 signalling. This figure summarizes schematically the signalling of the nod-like receptors nod 1 and nod 2. PAMP: Pathogen-associated molecular particles; IKK: IκB kinase; MAPK: Mitogen activated kinase; NF-κB: Nuclear factor κB; CARD: Caspase recruitment domain.

NLR family is defined by a common nucleotide binding domain and leucine-rich repeat series. All NLRs have a central ATPase region, which is called the NACHT domain^[32]. Until now 22 NLRs have been identified in humans. They differ from each other by heterogeneous N-terminal effector domains and can be divided into four subgroups. Class II transactivator (CIITA) is defined by an acidic transactivation domain (AD), and neuronal apoptosis inhibitor proteins (NAIPs) contain a baculovirus inhibitor of apoptosis protein repeat (BIR). The caspase recruitment domain (CARD) is common for NLRs, including nod 1 and nod 2 (schematic overview see Figure 2). Finally, NLRs share a pyridine domain (PYD). NLRs are important pattern recognition receptors in the intracellular compartment. Their activation leads to activation of innate immunity through nuclear factor-κB (NF-κB), mitogen-activated protein kinase (MAPK), and interferon regulatory factors (IRFs)^[33]. NLRs contribute to innate immunity by formation of the inflammasome. This complex is formed by NLRP1, NLRP2, NLRP3, and NLRC4 upon recognition of physical damage to the plasma membrane or certain pathogen-associated molecular particles (PAMPs). Inflammasome formation can directly lead to caspase 1 activation, or it can recruit the adaptor protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) to ac-

tivate caspase 1^[34]. Serving another means of controlling infection, nod 1 and nod 2 can induce autophagy through activation of LC3-positive speckles^[35]. In dendritic cells, nod 2-induced autophagy apparently plays a key role for bacterial elimination and antigen presentation^[36]. The role of NLR has been implied for some diseases. In patients with early-onset Crohn's disease, a frameshift mutation of the nod 2 gene has been found, while in early-onset sarcoidosis a gain of function mutation of the NACHT domain has been identified^[37]. There also is an indication for activation of NLRP3 in microglia in Alzheimer's disease by peptide amyloid-β^[38].

Role of TLRs and NLRs in HF

The role of the innate immune system in HF has been controversially discussed. Inflammation plays an important role in most cardiac diseases, and receptor-mediated innate immunity is primarily investigated with respect to TLRs. The role of innate immune cells and NLRs is also subject to current research. All known human TLRs have been found in the heart. However, until yet, the best-characterized TLR in cardiovascular diseases is TLR4 (Figure 3). Their expression level, however, varies greatly. Expression of TLR4, TLR3, and TLR2 is at least 10 times higher than that of any other TLR in the heart^[39]. Although TLRs were first known for their role in innate

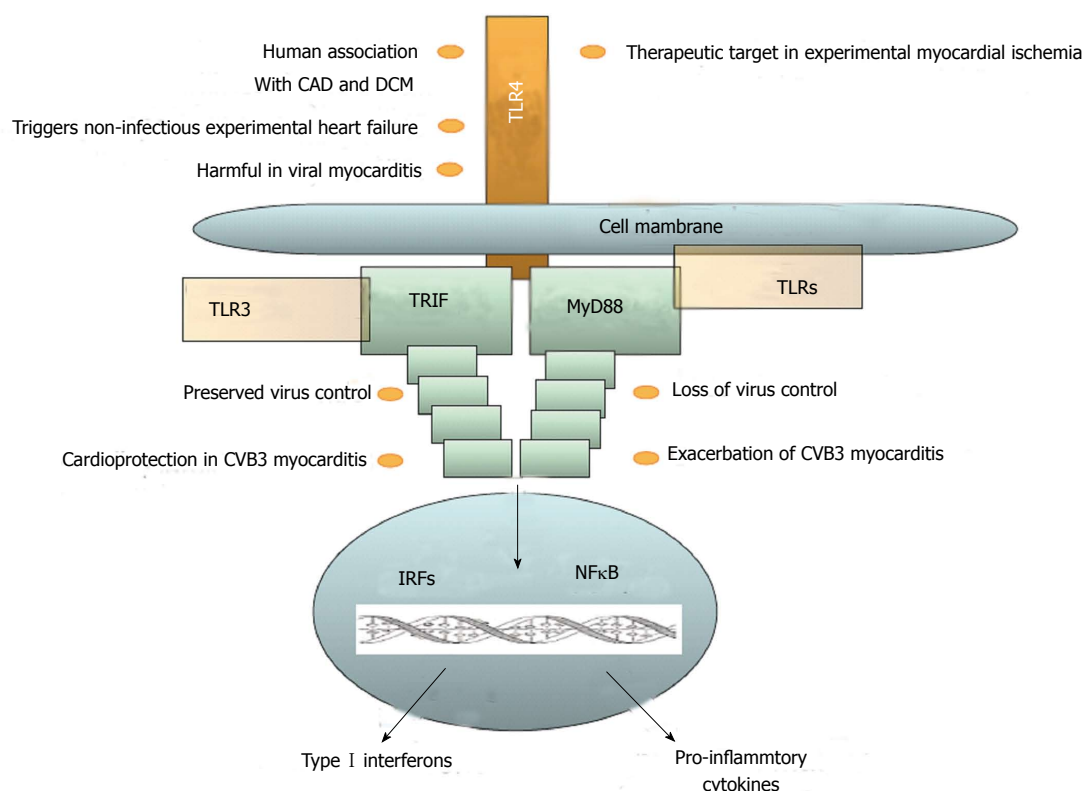


Figure 3 The role of Toll-like receptor 4 in heart failure. This figure summarizes current knowledge of the pathophysiological role of Toll-like receptor 4 (TLR4). CAD: Coronary artery disease; DCM: Dilated cardiomyopathy; TLR3: Toll-like receptor 3; TRIF: TIR domain-containing adaptor inducing interferon- β ; MyD88: Myeloid differentiation factor 88; IRF: Interferon regulatory factor; NF κ B: Nuclear factor kappa B.

immunity in their action against infection, inflammation in the heart is rarely caused by infectious agents. Other mechanisms lead to an inflammatory response, which often activates TLR pathways. Hemodynamic stress results in inflammation in the myocardium. Myocardial stress increases IL-6 production, which leads to an inflammatory response in the same manner as production of reactive oxygen species (ROS) due to mechanical strain. Macrophage infiltration is triggered by MCP-1 and TGF- β ^[40]. TNF- α is released by macrophages, mast cells, endothelial cells, and fibroblast. This secretion is triggered not only by infectious agents but also by tissue damage^[41]. Necrosis in the myocardium leads to distribution of intracellular particles, which in turn activates the innate immune system. ROS activates innate immune response, but also directly impairs cardiac function. DAMPs activate the complement system and TLRs at the same time^[42]. After activation of the TLR pathway, NF- κ B induces the expression of pro-inflammatory cytokines and chemokines in endothelial cells, fibroblasts, leukocytes, and vascular cells^[43]. Although research has disclosed little for the involvement of NLR in HF, studies have taken place on the effects of the inflammasome in the ischemia-reperfusion model. These results have revealed that mice deficient in caspase-1 or ASC have markedly reduced infarct formation, fibrosis, and cardiac dysfunction. It was further shown that inflammasome activation and IL-1 β production occurred primarily in cardiac fibroblasts and leukocytes. This leads to the conclusion that NLRs

do play a role in cardiac remodeling and may represent an interesting therapeutic target in the future^[34]. Various immune cells evolve to serve functions in primary immune response to tissue damage in the heart, but may also perform a key function in limiting inflammation. Recent investigations have begun to unravel the complex system of macrophage subspecies and functions.

MACROPHAGES

Until now, two different phenotypes have been defined. M1 macrophages are described as first line of defense, with their increased microbicidal capacity as well as production of pro-inflammatory cytokines. M2 macrophages show increased phagocytic activity: they secrete the anti-inflammatory IL-10 and express IL-1 receptor antagonist^[44]. The definition of just two subtypes is most likely oversimplified, and a functional perspective could prove more useful in distinguishing pro-inflammatory, regulatory, and reparative macrophages. The phenotype of macrophages is probably defined by a constantly changing variety of cytokines, chemokines, and growth factors, which enable great flexibility in the system^[45]. Regulatory T cells (Tregs) have also been reported to possibly influence the macrophage phenotype. These regulatory cells suppress inflammation through IL-10 and TGF- β secretion, or by cell-cell contact. Mice lacking CCR5—which thus reduces Treg infiltration - show increased inflammation and MMP activity^[46].

ISCHEMIA/REPERFUSION INJURY

To give some order to these many consequences of cardiac tissue damage, the example of ischemia/reperfusion injury can provide an overview of the immune response. Three phases can be determined that lead to adverse cardiac remodeling. First, neutrophils and pro-inflammatory macrophages migrate to the infarct site, with attraction by chemokines and cytokines secreted upon activation of innate immune pathways. Upon finishing the task of clearing the infarct site of necrotic cells, neutrophils go into apoptosis, which ends the inflammatory phase. Various macrophage subtypes migrate to the infarct in the proliferative phase. They activate endothelial cell growth and myofibroblast formation, with resultant production of a scar. In the final phase, more cells go into apoptosis and collagen cross-links, which possibly leads to ventricle dilation as the infarct matures^[47].

During recent decades, intensive research has led to better understanding of ischemia/reperfusion (I/R) injury. I/R injury leads to rapid activation of the immune system, which in turn results in increased expression of TNF, IL-1 β , IL-6, NO as well HSP^[48,49]. These and other factors lead to infiltration of the infarct with neutrophil granulocytes. In canine and mouse models, infiltration ceased after 3-7 d, and the neutrophils went into apoptosis^[50]. Early infiltration of the myocardium can cause more extensive cytotoxic injury to viable cardiomyocytes, which leads in turn to additional damage in the heart^[51]. ROS generated by neutrophils may contribute to that adverse effect, as well as interaction with cardiomyocytes through intercellular adhesion molecule-1 (ICAM-1) and integrin^[52]. To partially control the immune response, annexin and lactoferrin are transmitted by dying neutrophils to terminate further migration of neutrophils-but at the same time possibly attract macrophages to the site^[53]. Furthermore, TNF- α , released at the infarcted area by resident mast cells, also promotes mononuclear cell infiltration^[54]. These macrophages begin ingesting the apoptotic cells and, in turn, release cytokines as IL-10, TGF- β pro-resolving lipoxins, and resolvins^[55]. Upregulation of IL-10 and TGF- β suppress production of adhesion molecules. Another process for inhibition of leukocyte adhesion is carried out by endogenous integrin ligands of endothelial cells^[56]. Some experiments conducted on knock-out mice have provided further insights on the involved immune cells. The attempt to evade the effect of macrophage I/R injury was analyzed in monocyte chemoattractant protein (MCP) 1/CCL2 knock-out mice. MCP1 recruits pro-inflammatory and phagocytic macrophages to the infarct. Compared to wild-type mice, knock-out mice exhibited reduced dilative remodeling with equal infarct size^[57]. Similar results were achieved for IL-1-deficient mice. Although infarct size did not vary, the extent of cardiac remodeling was reduced in deficient mice compared to wild type^[58]. Those findings support the theory that the initial immune response does not pose the problem, but that long-term activation causes adverse effects. This could partly explain results for ICAM1-deficient mice.

Once again compared to wild type, they showed no difference in infarct size, even after 1-3 wk^[59]. The same applies to mice with ICAM1 and P-selectin deficiency. Neutrophil migration was decreased, but infarct size did not vary compared to wild type^[60]. These results could also suggest that the role of neutrophils has been overestimated. ROS is a mediator among others secreted by neutrophils. It can activate complements, stimulate P-selectin expression promoting cell migration, and upregulate chemokine and cytokine synthesis through the NF- κ B pathway^[61]. ROS, as well as ATP and potassium abundance, may activate the inflammasome. The inflammasome is expressed by border-zone cardiomyocytes, white blood cells in the granulation tissue, and cardiac fibroblasts. Inflammasome formation can be inhibited by P2X7 and cryopyrin, which leads to a decrease in infarct size^[62]. Research on TLRs involved in I/R-injury focusses mainly on TLR2 and TLR4. TLR2 seems to play a key role. TLR2 knock-out mice demonstrate better contractile function after I/R injury, and they show similar infarct size, but less ventricular remodeling compared to wild type. Fibrosis is reduced in the non-infarct area, and TGF- β and collagen type 1 expression are lower in knock-out mice. The recovery of LV-developed pressure is also better in TLR2-deficient mice^[63,64]. Further research has focused on the transmission of this effect to determine whether it entailed a central effect using TLR2 in the heart, or a peripheral effect involving white blood cells. Infarct size was compared for TLR2-deficient mice and wild-type mice with TLR2-deficient bone marrow. Infarct size did not differ significantly. When TLR2-deficient mice were injected with wild-type bone marrow, infarct size increased compared to purely TLR2-deficient mice. It was possible to inhibit this effect by administering an TLR2 antagonist-which resulted in smaller infarcts, enhanced overall cardiac function, and reduced inflammation and apoptosis^[65]. We and others investigated the role of TLR4 in myocardial infarction. TLR4-deficient mice displayed an improved outcome and decreased cardiac inflammation, as also revealed by others^[66,67]. Moreover, pharmacological inhibition of TLR4 using the antagonist eritoran led to beneficial effects, which suggests a potential new therapeutic strategy in myocardial ischemia, at least under experimental conditions^[68]. Mice deficient in TLR4 also showed smaller infarct size after I/R injury^[69]. Pre-treatment with LPS at 24 h before an I/R injury experiment results in better LV function compared to the sham group^[70]. TLR2-TIRAP signaling mediates this effect, in which GSK-3 β is subsequently inactivated-which prevents it from destabilizing mitochondria and leading to cell death^[71].

VIRAL CARDIOMYOPATHY

The role of the innate immune system in viral cardiomyopathy has been primarily established by experiments using mice infected with coxsackievirus B3 (CVB3). In humans it is known that cardiac CVB3 infection needs intact interferon-I signaling^[72]. TLR4-deficient mice exhibited higher titers of coxsackievirus B3 (CVB3) two

days after infection, but decreased titers and myocarditis in a 12-d follow up. The cytokines IL-1 β and IL-18 were reduced in TLR4-deficient mice^[73], Knock-out mice deficient for the TLR downstream adapter protein MyD88 are protected from CVB3 infection^[74]. Interestingly, we found in TRIF knock-out mice a much higher susceptibility to CVB3 infection when compared to wild-type mice: to include induction of mortality, loss of virus control, and exacerbation of pro-inflammatory cytokine expression in heart tissue^[75]. These data from MyD88 and TRIF knock-out mice suggest not only harmful effects of TLRs, but also cardioprotection in CVB3-induced myocarditis. TLR7 and TLR9 contribute to the susceptibility of MyD88-deficient mice in experimental myocarditis^[76]. This is also strengthened by our finding that shows that MyD88 may contribute to the modulation of TLR9 in CVB3-induced myocarditis in mice^[77]. In another study, infection of TLR3-deficient mice with encephalomyocarditis virus (EMCV)-a ssRNA virus-interestingly led to earlier death in knock-out mice, combined with increased viral replication and myocardial injury^[78]. The mRNA expression of TNF, IL-1 β , and IL6 was down-regulated, whereas IFN- β was up-regulated^[78]. IRAK-deficient mice and MyD88-deficient mice both exhibit lesser degrees of myocarditis and viral replication after infection, as well as improved survival. Levels of IFN- β were higher in MyD88 knock-out, and IFN- α and IFN- γ were increased in IRAK knock-out. Overall inflammation was reduced^[74,79]. Knock-out in cytokines/chemokines led to higher mortality, a greater extent of myocardial injury, higher viral titers for TNF knock-out and EMCV infection, as well as NO knock-out and CVB3 infection^[80,81].

DILATED CARDIOMYOPATHY

Activation of the immune system is widely considered a pathophysiological mechanism in DCM^[82-87]. For example, we disclosed that the initial white blood cell count upon initial hospital admission in DCM patients predicts long-term mortality in patients with DCM and severe LV dilation^[84]. In addition, genetic variants of TLR4 are significantly associated with cardiac recovery in DCM patients, which suggests a potential role of receptor-mediated innate immunity in this disease^[88]. Since TLRs are evidently involved in HF, and although viral or bacterial agents are much less frequently the cause than is ischemia, for example, it is interesting to examine a number of known DAMPs and their link to HF. For HSP60 and HSP70, a possible connection to HF has been evidenced. Both are increased in advanced HF. HSP60 trafficking through the plasma membrane is linked to apoptosis, and serum levels of HSP70 correlate with the severity of cardiac dysfunction^[89]. Decreased levels of TLR2 and TLR4 have been defined in all subgroups of cardiomyopathy, ischemic cardiomyopathy (ICM), dilated cardiomyopathy (DCM), and viral cardiomyopathy (VCM), whereas TIRAP and IRAK4 are up-regulated^[7]. A much wider overview of genetic alternations in cardiac disease allows fundamental compound analysis of innate immune sig-

naling genes. It has showed that the failing heart shows a different expression plot when compared to non-failing heart tissue. Further gene expression in viral cardiomyopathy and idiopathic dilated cardiomyopathy is similar and is distinguished from ischemic cardiomyopathy. This phenomenon suggests different immunological involvement of VCM and DCM compared to ICM, and supports the theory that DCM may evolve from VCM.

THERAPEUTIC IMPLICATIONS

Early studies on the influence of the inflammatory response in HF confirmed the harmful effects of methylprednisolone administration in patients with myocardial infarction^[90]. Since that time, many new options have evolved. Nevertheless, it is apparently no less difficult to achieve a positive result, even though current knowledge of innate immunity in HF is much more detailed. These difficulties are obvious in two studies on anti-TNF alpha therapy, with etanercept eventually proving not beneficial and even deleterious^[91,92]. Another problem may lie in the limited comparability of humans and animal models. Whereas, in a canine model, antibody-inhibiting leukocyte adhesion acted in a protective manner to limit infarct size by 40% to 50%, there was no effect on infarct size in humans with STEMI administration of CD11b/CD18 integrin receptor inhibition^[93,94]. There are, on the other hand, a number of promising substances. TLR4 antagonist eritoran significantly reduces infarct size^[68]. New variations of lipid A have been found. They bind to TLR4 but demonstrate reduced agonistic activity (CRX-527, lipad-Iva). TAK-242 also inhibits TLR4 signaling, yet until now its target remains unknown. Ibudilast (AV411) is another TLR 4 antagonist, one that suppresses pro-inflammatory cytokines such as TNF and IL-6. It may induce IL-10 and is currently under trial for opioid dependence. OPN-401, a viral protein-derived peptide, inhibits TLR4 signaling but is still in development. OPN-305 is a promising monoclonal antibody-inhibiting TLR2 and is currently in orphan status for prevention of I/R injury after organ transplantation. AP177-DNA aptamer binds to TLR and antagonizes TLR2 ligand binding^[95]. Anakinra, a IL1 receptor antagonist, suppresses post-infarct inflammation and has showed lower incidence of HF^[96]. In summary, although knowledge of the pathophysiology of the innate immune system in HF has substantially increased and new therapeutic targets have been addressed under experimental conditions, future investigations, especially clinical trials and experimental research in human tissue—are needed to develop effective innate immune system modulating treatment in HF.

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Atypical presentation of acute and chronic coronary artery disease in diabetics

Hadi AR Hadi Khafaji, Jassim M Al Suwaidi

Hadi AR Hadi Khafaji, Internal Medicine Department, Toronto General Hospital, Toronto University, Toronto, Ontario M5G 2C4, Canada

Jassim M Al Suwaidi, Qatar Cardiovascular Research Center and Heart Hospital Hamad Medical Corporation, Doha, Qatar

Author contributions: Khafaji HARH and Al Suwaidi JM contributed to organizing, writing and reviewing this article.

Correspondence to: Hadi AR Hadi Khafaji, FRCP^{Glasgow}, Internal Medicine Department, Toronto General Hospital, Toronto University, 200 Elizabeth Street, 14 EN-208 Toronto, Ontario M5G 2C4, Canada. hadi968@hotmail.com

Telephone: +1-647-4700994 Fax: +1-416-5955826

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Abstract

In patients with diabetes mellitus, cardiovascular disease is the principal cause of mortality and chest pain is the most frequent symptom in patients with stable and acute coronary artery disease. However, there is little knowledge concerning the pervasiveness of uncommon presentations in diabetics. The symptomatology of acute coronary syndrome, which comprises both pain and non-pain symptoms, may be affected by traditional risk factors such as age, gender, smoking, hypertension, diabetes, and dyslipidemia. Such atypical symptoms may range from silent myocardial ischemia to a wide spectrum of non-chest pain symptoms. Worldwide, few studies have highlighted this under-investigated subject, and this aspect of ischemic heart disease has also been under-evaluated in the major clinical trials. The results of these studies are highly diverse which makes definitive conclusions regarding the spectrum of atypical presentation of acute and even stable chronic coronary artery disease difficult to confirm. This may have a significant impact on the morbidity and mortality of coronary artery disease in diabetics. In this up-to-date review we will try to analyze the most recent studies on the atypical presentations in both acute and chronic

ischemic heart disease which may give some emphasis to this under-investigated topic.

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Key words: Diabetes mellitus; Acute coronary syndrome; Acute myocardial infarction; Ischemic heart disease; Atypical presentation; Silent myocardial ischemia

Core tip: Atypical presentations of both acute and chronic ischemic heart disease in diabetic patients is one of the most under-investigated subjects despite extensive research into coronary artery disease even in major clinical trials. To date, according to available data from numerous studies, the impact of atypical presentation on outcome is highly controversial making definitive conclusions difficult. This may have a significant impact on morbidity and mortality of acute and chronic coronary artery disease in diabetics.

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INTRODUCTION

Cardiovascular morbidity is the main cause of death in diabetics. It is predicted that 366 million patients globally will have diabetes mellitus by 2030. As diabetes mellitus progresses, it results in endothelial dysfunction and changes in energy metabolism which lead to atherosclerosis in medium- and large-caliber arteries, creating lesions in coronary, cerebrovascular and peripheral arteries. Additionally, atherosclerotic plaques tend to develop much earlier, advance more swiftly and are more diffuse in diabetic patients than in non-diabetics. These factors

contribute to a two to four-fold higher risk of cardiovascular events in diabetics compared to non-diabetics, with cardiovascular disease being the main cause of death. The combined mortality rate due to cardiovascular disease and diabetes mellitus is 245/100000 population for adults aged 30 to 70 years according to World Health Organization report^[1-3].

The overall frequency of coronary artery disease (CAD) among diabetics is 55%. To date, 90% of the published studies presenting data on the atypical presentation of chronic and acute ischemic heart disease are carried out in type 2 diabetics, while there are few data available on type 1 diabetics. Consequently, most of our conclusions in this review are for type 2 diabetes^[4-6].

Diabetic patients frequently present with silent myocardial ischemia (SMI), and the absence of an imperative clinical “warning symptom”. Statistics from the Framingham study showed that asymptomatic patients with various risk factors have an annual cardiac mortality rate of approximately 3%^[4,5,7]. Such outcomes from these studies raise numerous questions regarding diabetes mellitus and CAD: Why is myocardial ischemia repeatedly atypical or silent in diabetic patients? In what way is it discovered? What is its aftermath? How do we deal with it? The current analysis will tackle these issues. We identified studies *via* searches in MEDLINE, PubMed, EMBASE, and Current Contents and by reviewing reference lists in all the studies performed in the last 30 years from both developed and developing countries using the following keywords: diabetes mellitus, acute coronary syndrome (ACS), acute myocardial infarction (AMI), ischemic heart disease, atypical presentation, and SMI. We attempted to provide conclusions and future perspectives on this under-evaluated topic according to up-to-date studies from different parts of the world.

POSSIBLE EXPLANATION FOR THE ATYPICAL PRESENTATION OF ACUTE CORONARY SYNDROME IN DIABETICS AND THE PROGNOSTIC IMPLICATIONS

Chest pain is the cornerstone symptom of ACS. However, data concerning the prevalence of atypical presentation among these patients and its relation to subsequent care is scarce. CAD has specificities in diabetics with pervasive atherosclerosis. Diabetic patients are also more frequently asymptomatic, with a wide range of atypical presentations which makes the diagnosis of CAD challenging. In addition, diabetic patients with CAD have poorer outcomes than non-diabetics. CAD is the foremost source of morbidity and mortality in diabetic patients with higher mortality after an acute cardiac event compared to non-diabetics. Such inconsistencies may be related to the degree of CAD in diabetics, the magnitude of left ventricular remodeling, and the occurrence of significant ventricular dysrhythmias^[8-27].

Despite the fact that CAD is the primary vascular

complication of diabetes, there is a significant gap in our knowledge and understanding on atypical ACS symptoms in diabetics. Conventional risk factors, such as, hypertension, diabetes, hypercholesterolemia and smoking have a significant impact on the symptomatology of ACS and stable angina, including both pain and non-pain symptoms. Although numerous investigations on diabetes management have been performed, only a few studies have focused on atypical ACS symptoms in patients with diabetes with contradictory results. Diabetics may have a diminished awareness of ischemic chest pain which could result in an uncharacteristic presentation. This may be explained by autonomic neuropathy and prolongation of the anginal perceptual threshold^[28]. In addition, diabetic patients with SMI have evidence of a disseminated abnormality in metaiodobenzylguanidine uptake on positron emission tomography. A similar finding was also observed in asymptomatic diabetic patients on stress testing with a dipyridamole stress myocardial scan and contrast echocardiography in approximately 60% of diabetic patients, these findings reflect abnormal pain perception interrelated with sympathetic denervation^[29]. SMI is seen more frequently in diabetic patients than in the general population. SMI may be the main atypical presentation observed in major clinical trials compared to other forms of atypically presented CAD in both acute and chronic forms. However, the exact prevalence of SMI remains unidentified^[30]. In general, the frequency of silent CAD diverges according to the test used and the patient population investigated. The prevalence of silent CAD is 6%-23% in low-risk diabetics, and can be as high as 60% in high-risk diabetic patients. Recently it was recognized that silent CAD has a similar prognosis and adverse events rate when compared with symptomatic CAD^[31]. Possible explanations for the dissimilar symptoms in patients with diabetes mellitus, comprise central mechanisms such as altered thresholds of pain sensitivity, beta-endorphin levels, in addition to autonomic neuropathy resulting in sensory denervation. The American Diabetes Association states that patients with symptomatic autonomic neuropathy are at increased risk of sudden death; however, it still controversial whether there is adequate scientific data available to indicate that cardiac autonomic neuropathy contributes to silent ischemia and whether specific diabetic patients might gain benefit from routine testing for occult ischemia^[31].

In the last few years, diabetics have not experienced the same decline in CAD-related mortality as non-diabetics. The poor prognosis associated with diabetes after AMI has been witnessed in several studies despite adjustment for age, sex, coronary risk factors^[12,13,15-20] and associated comorbidities^[32]. Contradictory evidence is available concerning the morbidity and mortality of diabetic patients managed with insulin *vs* oral hypoglycemic agents or diet after AMI^[12,18,27,32,33]. Similarly, uncertainty still exists regarding the negative prognostic implications of diabetes in patients with a different spectrum of ACS *i.e.*, unstable angina, non-ST and ST-segment elevated AMI. It is imperative to establish whether these patients

are consistently receiving proven cardiac interventions under current practices.

SILENT MYOCARDIAL ISCHEMIA AS A MODE OF ATYPICAL PRESENTATION IN DIABETICS (TABLE 1)

Silent myocardial infarction/ischemia (SMI) is more frequent than formerly thought. Up to 25% of patients with CAD have suffered silent SMI; the magnitude of the myocardium affected is on average 10% of the left ventricle muscle mass, and it is more prevalent in diabetics. The phenomenon of SMI is still debatable. The presence of cardiac autonomic dysfunction is the assumed factor that influences the frequency of SMI in diabetics^[34]. Hence, the importance of identifying individuals with a high risk for cardiovascular events, prior to symptom onset may be of significance. Diabetes mellitus affects vascular endothelium, causing endothelial dysfunction^[35]. A study assessed the frequency, scope, and independent predictors of SMI in 2 large independent cohorts of consecutive patients without a history of MI referred for rest/stress myocardial perfusion single photon emission computed tomography. One thousand six hundred and twenty-one patients were registered in the derivation cohort and 338 patients in the validation cohort. SMI was diagnosed in patients with a myocardial scar involving $\geq 5\%$ of the left ventricle. In the derivation cohort, 23.3% had SMI. The median infarct size was 10% [interquartile range (IQR) 5%-15%] of the left ventricle. The occurrence of SMI was 28.5% in diabetics *vs* 21.5% in non-diabetics ($P = 0.004$). Diabetes mellitus was an independent predictor for the presence of SMI (OR = 1.5; 95%CI: 1.1-1.9; $P = 0.004$). In the validation cohort, the prevalence of SMI was 26.3%, with a higher incidence in diabetics (35.8%) compared to non-diabetics (24%; $P = 0.049$). The median infarct size was 11.8% (IQR, 5.9%-17.6%) of the left ventricle. After logistic regression analysis; diabetes mellitus was a noteworthy prognosticator of the presence of SMI confirming the derivation cohort result^[36].

In a cross-sectional study involving 200 subjects (mean age; 46 ± 10 years, 31 had diabetes), the subjects underwent an exercise stress test. A positive test for silent ischemia was seen in 19% of diabetics and 13% of non-diabetics, which was not statistically significant ($P = 0.397$). Hypertension and obesity were found more frequently in diabetics (48% *vs* 27% and 35% *vs* 18%, respectively)^[37]. Blood lipid levels may predict SMI in non-insulin dependent diabetes. A study included 220 asymptomatic diabetics who underwent laboratory tests and gated single-photon emission computed tomography with coronary angiography as the confirmatory test, when gSPECT detected ischemia. A higher level of total cholesterol was seen in gSPECT-positive diabetics, together with low-density lipoprotein (LDL), and triglycerides ($P < 0.05$). High-density lipoprotein (HDL) levels were lower in this group ($P < 0.05$). HDL was the most important normalized variable. This study included more men (33.3%) than

women (24.8%). HDL levels were significantly lower in these patients. The association between low HDL and high triglycerides was a strong indicator of myocardial ischemia in type 2 diabetics without clinical cardiovascular signs^[38]. A gated myocardial perfusion SPECT in asymptomatic diabetics with a high combination of cardiovascular risk factors detected SMI in a significant proportion of patients and this seemed to be related to future coronary events. Diabetic nephropathy may indicate a greater likelihood of abnormal studies^[39].

A study evaluated the pervasiveness of SMI in 147 subjects in a diabetic Afro-Caribbean population. 23.1% had SMI; these patients had a personal history of cardiovascular disease similar to those without diabetes. On multivariate logistic-regression analyses, the adjusted odds ratio of SMI was considerably higher in patients with a personal history of cardiovascular disease (4.36, 95%CI: 1.36-13.96; $P = 0.01$) and left ventricular hypertrophy (LVH) (2.46, 95%CI: 1.03-5.86; $P = 0.04$)^[40].

Dobutamine stress echocardiography may be a useful diagnostic test for detecting SMI, especially in diabetic patients at high cardiovascular risk. A study of 79 diabetics (average age = 58.8 ± 11.8 years) revealed that 67.1% had a positive test, with a predominance of motion abnormalities in the anterior area (83%). Microalbuminuria ($P = 0.0001$), inactivity ($P = 0.0001$), dyslipidemia ($P = 0.0002$), arterial hypertension ($P = 0.001$), smoking (0.003) and male sex ($P = 0.004$) were the main cardiovascular risk factors associated with positivity^[41].

In the detection of ischemia in asymptomatic diabetics (DIAD) study, the largest prospective study with a 4.8-year follow-up period included 1123 asymptomatic persons with type 2 diabetes who were randomized to either testing with stress myocardial perfusion scan or no testing. In this study, 53%-75% of participants with intermediate to high cardiovascular risk had a prevalence of inducible ischemia on screening that ranged from 21% to 24%, which was almost comparable to lower-risk patients (19%-23%). Patients with intermediate-/high-risk had higher rates of cardiac events (only significant for the UKPDS risk engine 4.2 *vs* 1.2%, $P = 0.002$). The yearly cardiac event rate was $< 1\%$ in all risk groups, apart from the high-risk UKPDS group (approximately 2% per year). Surprisingly the annual cardiac event rate for intermediate/high risk was low and not altered by standard testing for inducible ischemia^[42].

High LDL level and higher carotid intima-media thickness are predominant issues that can indicate whether a patient with non-insulin dependent diabetes (NIDDM) is at risk of SMI. A high carotid intima-media thickness is a substitute and dependable indicator of higher risk of CAD in non-insulin dependent diabetic patients, even in those without evident CAD^[43].

Another study determined SMI in 90 unselected middle-aged asymptomatic NIDDM patients (48 men; mean age: 49 ± 6 years, mean diabetes duration of 4 ± 4.2 years (range 1-21 years) without CAD as documented by treadmill exercise test. Four percent of patients had a positive test. Diabetics with SMI were older (55 ± 3

years vs 49 ± 6 years, $P = 0.04$), had a higher fibrinogen level (372 ± 51 vs 307 ± 71 mg/dL, $P = 0.04$) and had lower total exercise time and peak workload (375 ± 30 s vs 474 ± 115 s, $P = 0.04$; 7.3 ± 0.5 vs 8.9 ± 1.9 , $P = 0.04$, respectively). Insulin resistance is related to different atherosclerosis risk factors. Exercise test outcomes showed increased cardiac sympathetic activity and parasympathetic withdrawal in increased insulin resistance^[44]. Left atrial surface area independently predicted SMI after adjustment for established echocardiographic and inflammatory risk factors in diabetics^[45]. Age and differential pulse pressure may be predictors of SMI^[46].

A study estimated the frequency of SMI in 353 asymptomatic Caucasian diabetic patients using the treadmill test with single-photon emission computed tomography and exercise testing or dipyridamole injection with coronary angiography as the confirmation test. Patients with SMI (8.5% were diabetics: 3 IDDM and 13 NIDDM) were older and had autonomic neuropathy, hypertension, dyslipidemia and higher microalbuminuria (613 ± 211 mg/d vs 72 ± 245 mg/d; $P < 0.05$)^[47].

SMI may occur in more than 20% of asymptomatic patients with NIDDM. Conventional and evolving cardiac risk factors were not linked with abnormal stress tests, even though cardiac autonomic dysfunction was a resilient prognosticator of ischemia using adenosine technetium-99m sestamibi single-photon emission-computed tomography myocardial perfusion imaging in asymptomatic NIDDM patients and testing the efficiency of current American Diabetes Association screening guidelines. A total of 1123 patients, with no known or suspected CAD were randomly assigned to either stress testing and 5-year clinical follow-up or to follow-up only. In this study 22% had SMI; the strongest prognosticators for abnormal tests were abnormal Valsalva, male sex, and diabetes duration, but not traditional cardiac risk factors or inflammatory and prothrombotic markers. Choosing only patients who met the American Diabetes Association guidelines failed to detect 41% of patients with SMI^[48]. Erectile dysfunction may become a possible indicator to identify diabetic patients with SMI during screening, particularly in patients with additional cardiovascular risk factors^[49]. However, diabetics may have a higher prevalence of angina pectoris during daily activity than non-diabetics^[50]. Using dobutamine stress echocardiography to detect SMI, significant CAD was identified in 9% of asymptomatic diabetics. Dynamic left ventricular outflow obstruction was detected in 59% of diabetics and in only 22% of non-diabetics, however, these results need to be investigated in future studies^[51].

The association between SMI and cardiac autonomic neuropathy has been reported in a few studies (Table 1). Autonomic dysfunction is seen in 85.7% of diabetics with SMI vs 18.7% of diabetics without silent ischemia ($P = 0.001$). The incidence of SMI was higher in patients with autonomic neuropathy (40% vs 10%) $P < 0.001$. The duration of diabetes was greater (13 ± 1.59 years) in patients with autonomic neuropathy, and systolic blood pressure was predictive of silent ischemia in diabet-

ics^[52-54].

A few other studies^[55-65] assessed different aspects of the association between SMI and diabetes (Table 1). Patients with SMI had higher ischemia in the working forearm compared to diabetic patients with and without neuropathy. There is a quantitative and qualitative difference in ischemic tolerance between patients with SMI and patients with diabetic neuropathy^[57,58]. The role of beta endorphin in diabetic patients with SMI may be less substantial than in non-diabetics; therefore, diabetic neuropathy which affects the autonomic pain fibers that innervate the heart may be involved in the pathogenesis of SMI in diabetics and appears to be the most probable reason for the absence of pain^[59,60].

ATYPICAL PRESENTATION OF ACUTE CORONARY SYNDROME IN DIABETICS

Many reports including major clinical trials and sporadic studies (Tables 2 and 3) have shown that diabetes mellitus is an independent predictor of atypical presentation of ACS with a controversial outcome^[66]. Several studies reported that diabetic patients had less pain compared to non-diabetics^[67-75], while other studies found no difference^[76-81].

Studies which have shown diabetes mellitus is a predictor of the atypical presentation of acute coronary syndrome (Table 2)

In a nation-wide survey conducted in 2133 consecutive ACS patients who were separated into three age subgroups: < 65 years ($n = 974$), $65-74$ years ($n = 500$), and ≥ 75 years ($n = 639$), the incidence of no anginal pain/atypical symptoms on presentation increased with age in all ACS patients (14%, 21%, and 32%, in the three age subgroups, respectively; $P < 0.0001$). The occurrence of ST-elevation on admission electrocardiogram decreased with advancing age (59%, 46%, and 42%, in the three age subgroups, respectively; $P < 0.0001$), while ST-depression progressively increased (14%, 24%, and 28%, respectively; $P < 0.0001$). In a multivariate analysis, variables linked with no anginal pain/atypical symptoms on presentation were: history of heart failure, age, lack of past angina, diabetes, and non-smoking. ST-elevation was inversely associated with no anginal pain/atypical symptoms on admission (OR = 0.48; 95%CI: 0.37-0.63)^[68].

A study by Culić *et al.*^[69] who performed subgroup analyses showed that diabetes was an independent prognosticator of "atypical" presentation of AMI in women. In this prospective, observational study of a large number of symptoms in 1996 patients, it was established that chest pain was more often reported by males, smokers, and hypertensive, non-diabetic, and hypercholesterolemic patients. Women frequently reported non-chest pain other than epigastric and right shoulder pain, along with a range of non-pain symptoms. The independent predictors of atypical AMI presentation in both men and women were diabetes mellitus ($P = 0.0002$ and $P = 0.002$,

Table 1 Studies on silent myocardial ischemia as a mode of atypical presentation in diabetics

Ref.	Study population	Study type/country	Silent ischemia %	Conclusion
Arenja <i>et al</i> ^[36]	1621 pts in the derivation cohort + 338 pts in the validation cohort	Derivation cohort/Switzerland	23.3%-28.5% in DM and 21.5% in non-DM	DM is an independent predictor for the presence of SMI (OR = 1.5; 95%CI: 1.1-1.9, <i>P</i> = 0.004). In the validation cohort, the prevalence of SMI = 26.3% (<i>n</i> = 89), while the prevalence in diabetics (35.8%) <i>vs</i> non-diabetics was 24% (<i>P</i> = 0.049)
Sheikh <i>et al</i> ^[37]	200 subjects, 31 diabetics <i>vs</i> 169 non-diabetics	A cross-sectional study/Pakistan	(19%) diabetics <i>vs</i> (13%) non-diabetics	No significant difference in the frequency of SMI in diabetics <i>vs</i> non-diabetics
Peña <i>et al</i> ^[38]	220 asymptomatic NIDDM patients	A prospective, observational, analytical study/Havana	29.10%	Type 2 diabetics with ischemia had ↑ levels of total cholesterol, LDL and triglycerides. HDL levels were significantly ↓. The association of ↓ HDL with ↑ triglycerides was a strong indicator of SMI in NIDDM patients
Ruano Pérez <i>et al</i> ^[39]	56 asymptomatic diabetics	retrospective study	46.40%	Moderate-severe ischemia in 10.7%, necrosis with ischemia in 5.4% and necrosis in 7.1%, diabetic nephropathy was the only factor related to an abnormal SPECT (<i>P</i> = 0.043)
Blanchet Deverly <i>et al</i> ^[40]	147 NIDDM patients	cross-sectional study/France	23.10%	Multivariate logistic-regression analyses, the adjusted OR of SMI significantly ↑ in patients with a history of cardiovascular disease (4.36, 95%CI: 1.36-13.96, <i>P</i> = 0.01) and LVH (2.46, 95%CI: 1.03-5.86, <i>P</i> = 0.04)
Mbaye <i>et al</i> ^[41]	79 diabetics	Prospective/France	67.10%	Predominance of motion abnormalities in the anterior territory (83%). Cardiovascular risk factors associated with positivity of the test were microalbuminuria (<i>P</i> = 0.0001), inactivity (<i>P</i> = 0.0001), dyslipidemia (<i>P</i> = 0.0002), arterial hypertension (<i>P</i> = 0.001), smoking (0.003) and male sex (<i>P</i> = 0.004)
Bansal <i>et al</i> ^[42]	1123 NIDDM patients	Prospective/Detection of Ischemia in Asymptomatic Diabetics (DIAD) /United States and Canada (DIAD) study	21%-24% in the intermediate high risk group 19%-23% in the low risk group	Cardiac event rates ↑ in intermediate/high-risk. The annual cardiac event rate was ≤ 1% in all risk groups. In intermediate-/high-risk participants randomized to screening <i>vs</i> no screening, 4.8-yr cardiac event rates were similar (2.5%-4.8% <i>vs</i> 3.1%-3.7%)
Agarwal <i>et al</i> ^[43]	77 NIDDM	Prospective study/India	28.90%	The prevalence of SMI similar in males and females. Serum LDL levels > 140 mg % had a significant correlation with the prevalence of silent CAD (<i>P</i> = 0.04). The difference in CCA-IMT values was found to be statistically significant between the silent CAD and non-CAD groups (<i>P</i> = 0.019)
Ugur-Altun <i>et al</i> ^[44]	90 asymptomatic NIDDM patients	Prospective/Turkey	4%	Diabetics with SMI had ↑ fibrinogen level (372 ± 51 mg/dL <i>vs</i> 307 ± 71 mg/dL, <i>P</i> = 0.04), had ↓ total exercise time and peak workload (375 ± 30 s <i>vs</i> 474 ± 115 s, <i>P</i> = 0.04; 7.3 ± 0.5 <i>vs</i> 8.9 ± 1.9, <i>P</i> = 0.04, respectively)
Chico <i>et al</i> ^[47]	353 NIDDM asymptomatic Caucasians	Prospective/Spain	8.50%	SMI patients were older, had ↑ prevalence of autonomic neuropathy, microalbuminuria, hypertension, and dyslipidemia than those without
Wackers <i>et al</i> ^[48]	1123 NIDDM patients	Prospective/United States	20%	Predictors for abnormal tests: abnormal Valsalva, male sex and diabetes duration (5.2). Traditional cardiac risk factors or inflammatory and prothrombotic markers were not predictive. Ischemic adenosine-induced ST-segment depression with normal perfusion in women
Falcone <i>et al</i> ^[50]	618 patients with CAD	Prospective/Italy	58%	SMI during exercise seen in 58% of diabetics and 64% of nondiabetics. Both diabetics and non-diabetics with exertional SMI had ↑ heart rate values (<i>P</i> < 0.01), SBP (<i>P</i> < 0.01), rate-pressure product (<i>P</i> < 0.001), work load (<i>P</i> < 0.01) and maximum ST depression at peak exercise (<i>P</i> < 0.05)
Coisne <i>et al</i> ^[51]	49 diabetics and 63 non-diabetics	Prospective/France	9%	Significant CAD detected in 9% of asymptomatic diabetics. Dynamic left ventricular obstruction observed in 59% of the diabetic population and in only 22% in the non-diabetic population
Sukhija <i>et al</i> ^[53]	30 diabetics/30 non diabetics	Prospective/India	46.70%	Diabetics had ↑ heart rate and a greater number of supraventricular and ventricular ectopics, ↑ prevalence of multi-vessel involvement and diffuse disease compared to controls. 50% of diabetics and none of the controls had autonomic dysfunction. Autonomic dysfunction was present in 85.7% of diabetics with SMI <i>vs</i> 18.7% of diabetics without SMI (<i>P</i> = 0.001)
May <i>et al</i> ^[54]	240 diabetics	Prospective/Denmark	13.50%	Frequency of SMI did not differ significantly between diabetics and non-diabetics. Systolic blood pressure was predictive of SMI in diabetes
Tamez-Pérez <i>et al</i> ^[55]	60 NIDDM patients	Prospective/ Spain	17%	In a 2-yr follow-up, 4 diabetics developed symptomatic angina pectoris

Ahluwalia <i>et al</i> ^[56]	20 male diabetics	Prospective/India	50%	On exercise testing in diabetics, SMI was detected in 64% of the patients with 3 vessel disease, 50% of the patients with 2 vessel disease and 20% of the patients with one-vessel disease <i>vs</i> 18% of non-diabetic patients with three-vessel disease ($P < 0.05$) and in none of the patients with two- or one-vessel disease
Tanaka <i>et al</i> ^[61]	92 NIDDM patients	Prospective / Japan	38%	Diabetics with positive treadmill test were smokers, and had hypertension and ↑ triglyceride level compared to treadmill negative diabetics
Nesto <i>et al</i> ^[62]	30 diabetics with peripheral vascular disease	Prospective /United States	57%	57% had thallium abnormalities, with reversible thallium defects compatible with ischemia in 47% and evidence of prior, clinical SMI in 37%. Thallium abnormalities were seen more frequently in diabetics with concomitant hypertension and cigarette smoking ($P = 0.001$)
Koistinen <i>et al</i> ^[63]	136 diabetic subjects	Controlled study/Finland	29%	Coronary angiography of 34 diabetics; 12 had significant coronary artery narrowing; seven had unimportant atherosclerosis; 15 had patent coronary arteries
Theron <i>et al</i> ^[64]	52 IDDM and 87 NIDDM subjects	Prospective /South Africa	See conclusion	No statistically significant relationship between any parameter and the presence of autonomic neuropathy. Atypical infarctions not limited to subjects with autonomic neuropathy, the incidence much ↑ than the general population
Touze <i>et al</i> ^[65]	50 black African diabetics	Prospective /Africa	10%	SMI was ↓ among black African diabetics compared with white diabetics. The coronary lesions were mostly limited. Proximal narrowing and one-vessel disease mostly encountered-

↑: Increase/higher; ↓: Decreased/lower. CAD: Coronary artery disease; IDDM: Insulin dependent diabetes mellitus; NIDDM: Non-insulin dependent diabetes mellitus; MI: Myocardial infarction; HDL: High density lipoprotein; LDL: Low density lipoprotein; SMI: Silent myocardial ischemia/infarction; CCA-IMT: Common carotid artery intimal medial thickness

respectively), lower creatine kinase-MB fraction level ($P < 0.0001$ and $P = 0.0003$, respectively), older age ($P = 0.001$ and $P = 0.01$, respectively), and absence of smoking in men ($P = 0.005$). The independent predictors of non-pain symptoms in both men and women were higher levels of creatine kinase-MB fraction ($P = 0.01$ and $P = 0.049$, respectively) and diabetes mellitus ($P = 0.048$ and $P = 0.005$, respectively), while hypercholesterolemia ($P = 0.01$) in men was the predictor of atypical presentation^[69].

A recent study in South Korea evaluated the risk factors associated with atypical presentation according to age. In this study, diabetes and hyperlipidemia predicted atypical symptoms in the younger (< 70 years) age group. Comorbid illnesses such as stroke or chronic obstructive pulmonary disease were positive predictors in the older (> 70 years) age group^[70].

Statistics from a prospective clinical trial of patients with symptoms indicating ACS in 10 United States hospitals during emergency assessment compared patient demographics, clinical variables, and outcomes. Of 10783 subjects, a definitive diagnosis of long-established ACS was made in 24% of patients, of which 35% had AMI and 65% had unstable angina. Sixty-two percent of ACS patients and 9.8% of AMI patients had no pain. Patients with painless ischemia were older, and more frequently females with more cardiac and related diseases. Patients with painless AMI were less likely to be admitted to critical care units. Among patients with acute infarction, logistic regression predicting lack of pain categorized age, heart failure and diabetes as the main predictors with only age and heart failure in those with ACS. After controlling for clinical features, silent acute ischemia predicted augmented hospital mortality^[72].

In the National Registry of Myocardial Infarction 2 (NRFMI 2): a prospective observational study in the United

States, which included 434877 patients with MI, 33% had no chest pain on presentation to the hospital and were 7 years older than those with chest pain (74.2 years *vs* 66.9 years), more likely to be female (49.0% *vs* 38.0%), have diabetes mellitus (32.6% *vs* 25.4%) or previous cardiac failure (26.4% *vs* 12.3%) and have delayed presentation (mean, 7.9 *vs* 5.3 h). These patients were less likely to be diagnosed with SMI and were less likely to undergo thrombolysis or primary angioplasty (25.3% *vs* 74.0%), and treatment with aspirin (60.4% *vs* 84.5%), beta-blockers (28.0% *vs* 48.0%), or heparin (53.4% *vs* 83.2%). SMI patients had higher in-hospital mortality compared to symptomatic patients (23.3% *vs* 9.3%)^[73,74].

Many sporadic studies from different parts of the world both in developed and developing countries have assessed the atypical presentation of ACS in different communities. Such studies have shown diverse results (Table 2). A study assessed 9509 healthy adults over 5 years who had an average annual incidence of 3.6/1000 persons with unrecognized infarcts and 5.3/1000 persons with clinical infarcts. Patients whose electrocardiograms were initially read by cardiologists as non-infarcts, but by the computer as infarcts, had a high rate of unrecognized infarcts in the subsequent 5 years and a markedly higher 7-year mortality rate in the unrecognized infarct group *vs* the non-infarct population, but significantly lower than those who developed a clinical infarct. In this study, age, left axis deviation, left ventricular hypertrophy, cigarette smoking, systolic or diastolic blood pressure, and peripheral vascular disease were significant risk factors for unrecognized myocardial infarction on multivariate analysis. Cholesterol, diabetes, anxiety, and psychosocial problems, do not play a significant role in unrecognized infarcts^[75].

The Global Registry of Acute Coronary Events (GRACE study), is the largest multinational, prospective,

Table 2 Studies which have shown that diabetes mellitus is a predictor of atypical presentation of acute coronary syndrome

Ref.	Study population/	Study type/country	Atypical presentation %	Conclusion
Stern <i>et al</i> ^[68]	2113 ACS patients	Nationwide survey/ Israel	21.7% had no chest pain	In multivariate analysis, variables associated with no anginal pain/atypical symptoms on presentation (in ↓ order): history of heart failure, age, no past angina, diabetes and non-smoking. 18.7% of male patients had no chest pain on presentation vs 29.7% of females
Culić <i>et al</i> ^[69]	1996 MI patients	A prospective, observational study/ Croatia	14.8% had no chest pain	The independent predictors of atypical presentation in both gender; ↓ levels of CK-MB fraction ($P < 0.0001$ and $P = 0.0003$, respectively), NIDDM ($P = 0.0002$ and $P = 0.002$, respectively), older age ($P = 0.001$ and $P = 0.01$, respectively), and no smoking in men ($P = 0.005$) The independent predictors of the presence of non-pain symptoms; DM ($P = 0.048$ and $P = 0.005$, respectively), ↑ levels of CK-MB ($P = 0.01$ and $P = 0.049$, respectively) and hypercholesterolemia ($P = 0.01$) in both men and women A logistic regression analysis after adjustment for gender and ACS type indicated that diabetes and hyperlipidemia significantly predicted atypical symptoms in younger patients Less chest pain in diabetics vs non-diabetics ($P = 0.02$) No difference in pain intensity in diabetics with MI vs non-diabetics ($P \geq 0.05$) Diabetics with UA or MI were more likely to report mid-sternal chest pain ($P = 0.04$) and chest pain that radiated to the back of the left arm ($P = 0.01$) than non-diabetics Diabetics with UA or MI reported more SOB (53.1% vs 31.3%; NS) In diabetics with UA or MI, SOB was a factor in deciding to seek care
Hwang <i>et al</i> ^[70]	931 newly diagnosed as ACS	Retrospective/ South Korea	7.8% of younger pts and 13.4% of older pts	A logistic regression analysis after adjustment for gender and ACS type indicated that diabetes and hyperlipidemia significantly predicted atypical symptoms in younger patients
MacKenzie <i>et al</i> ^[71]	64 (12 women with DM)	Descriptive, cross-sectional/ Canada	See conclusion	Less chest pain in diabetics vs non-diabetics ($P = 0.02$) No difference in pain intensity in diabetics with MI vs non-diabetics ($P \geq 0.05$) Diabetics with UA or MI were more likely to report mid-sternal chest pain ($P = 0.04$) and chest pain that radiated to the back of the left arm ($P = 0.01$) than non-diabetics Diabetics with UA or MI reported more SOB (53.1% vs 31.3%; NS) In diabetics with UA or MI, SOB was a factor in deciding to seek care
Coronado <i>et al</i> ^[72]	2541 (1058 women, 410 women with DM);	Secondary analysis of multisite a prospective clinical trial/ United States	6.2% of patients with ACS and in 9.8% of AMI.	DM independent predictor of painless presentation in acute MI, but not in the ACS group. Diabetes more common in non-pain ACS (35% vs 26%; $P = 0.01$) Shortness of breath most common in the painless presentation group (72%) and women were more likely to have painless ACS (53%) ($P = 0.007$)
Vaccarino <i>et al</i> ^[73]	384878 patients	Prospective, observational study/ National Registry of MI/ United States	33%	Atypical presentation patient: older, ↑ proportion of women and diabetics without a significant interaction between sex and diabetes ($P = 0.30$). HF comorbidities and less likely to have coronary intervention with ↓ chance of anticoagulants, aspirin and β blocker usage
Canto <i>et al</i> ^[74]	434877 MI pts June 1994-March 1998	Prospective observational study United States	33% had no chest pain	Patients without chest pain on presentation: Likely to be diabetics (32.6% vs 25.4%) Older (74.2 yr vs 66.9 yr). Likely to be female (49.0% vs 38.0%) Likely to have prior HF (26.4% vs 12.3%) Had a longer delay before hospital presentation (mean, 7.9 h vs 5.3 h) Less likely to be diagnosed with confirmed MI at the time of admission (22.2% vs 50.3%) Less likely to receive thrombolysis or PCI (25.3% vs 74.0%), aspirin (60.4% vs 84.5%), BB (28.0% vs 48.0%), or heparin (53.4% vs 83.2%). 23.3% in-hospital mortality vs 9.3% in patients with chest pain By multivariate analysis, age, left axis deviation, LVH, cigarette smoking, systolic or diastolic BP, and PVD were the most significant risk factors. Cholesterol, DM, anxiety, and psychosocial problems, do not play a significant role in unrecognized MI
Medalie <i>et al</i> ^[75]	9509 healthy adult subjects	Israeli Heart Attack study, cohort/ Israel	3.6 unrecognized MI/ 1000 persons and 5.3 clinical MI/1000 persons	23.8% not initially recognized as having an ACS, < 33% of the population with atypical symptoms were diabetics. Less likely to receive effective cardiac medications ↑ hospital morbidity and mortality (13% vs 4.3%, respectively; $P < 0.0001$) ↑ hospital mortality rates in patients with presenting symptoms of pre-syncope/syncope. Nausea or vomiting, dyspnea and in those with painless presentations of UA
Brieger <i>et al</i> ^[76]	20881 ACS patients	Global Registry of Acute Coronary Events/multinational, prospective, observational study (in 14 countries)	8.4% presented without chest pain	23.8% not initially recognized as having an ACS, < 33% of the population with atypical symptoms were diabetics. Less likely to receive effective cardiac medications ↑ hospital morbidity and mortality (13% vs 4.3%, respectively; $P < 0.0001$) ↑ hospital mortality rates in patients with presenting symptoms of pre-syncope/syncope. Nausea or vomiting, dyspnea and in those with painless presentations of UA

↑: Increase/higher; ↓: Decreased/lower. MI: Myocardial infarction; UA: Unstable angina; AMI: Acute myocardial infarction; ACS: Acute coronary syndrome; DM: Diabetes mellitus; SOB: Shortness of breath.

Table 3 Studies which have not shown that diabetes mellitus is a predictor of atypical presentation of acute coronary syndrome

Ref.	Study population/	Study type/country	Atypical presentation %	Conclusion
Meshack <i>et al</i> ^[77]	589 patients, aged 25 to 74 yr, with AMI	A community-based surveillance program/ United States	Sweating (64.2%), fatigue (62.6%), dyspnea (60.3%), and arm or jaw pain (58.2%).	Adjusting for age, DM, gender, and relative to non-Hispanic whites, Mexican Americans were more likely to report chest pain, upper back pain, and palpitations, and less likely to report arm or jaw pain
Richman <i>et al</i> ^[78]	216 (19 women with DM); AMI	A prospective, observational study/ United States	No statistical difference in diabetics vs non-diabetics in terms of the presence chest pain	No difference in the frequency of chest pain or associated symptoms by diabetic status ($P \geq 0.05$); No chest pain symptoms was more common in diabetic patients (NS)
Kentsch <i>et al</i> ^[79]	1042 (330 women; 155 women with DM) with STEMI	Secondary analysis of MITRA PLUS (18786 pts.; North German Registry, NGR, 1042 pts.)/ Germany	16.9% of DM and 15.0% of non-DM	No difference in the frequency or intensity of chest pain by diabetic status Patients with DM reported significantly more dyspnea than those without DM (29.5% <i>vs</i> 19.5%; $P < 0.01$)
DeVon <i>et al</i> ^[80]	100 (50 women, 23 women with DM); DM	pective secondary analysis; descriptive, cross-sectional; structured interview/United States	3%	No difference in the frequency and severity of chest pain in diabetics vs non-diabetics ($P \geq 0.05$) No differences in UA symptoms by diabetic status Patients with DM reported weakness as the second most common symptom and more likely to describe chest pain as squeezing ($P = 0.02$) or aching ($P = 0.04$) than non-diabetics Diabetics had \uparrow frequency of hyperventilation ($P = 0.04$) and \downarrow frequency of nausea ($P = 0.04$) than non-diabetics
Thuresson <i>et al</i> ^[81]	N = 1939 (480 women, 82 women with DM)	Descriptive, cross-sectional study/ Sweden	See conclusion	No difference in chest pain or other ACS symptoms by DM status Women reported more tiredness/weakness, anxiety/fear, vomiting, back pain, left arm pain and neck or jaw pain than men ($P = 0.01$).

\uparrow : Increase/higher; \downarrow : Decreased/lower. STEMI: ST elevation myocardial infarction; UA: Unstable angina AMI: Acute myocardial infarction; ACS: Acute coronary syndrome; DM: Diabetes mellitus; PVD: Peripheral vascular disease.

observational study and involves 14 countries (Argentina, Australia, Austria, Belgium, Brazil, Canada, France, Germany, Italy, New Zealand, Poland, Spain, the United Kingdom, and the United States). Of the 20881 patients included, 8.4% had no chest pain, and 23.8% were not initially recognized as having ACS. These patients had higher hospital morbidity and mortality (13% *vs* 4.3%, respectively; $P < 0.0001$) and were less likely to receive effective cardiac medications than patients with typical presentation. After adjusting for potentially confounding variables, excluding diaphoresis, higher in-hospital mortality rates were seen in patients who presented with pre-syncope/syncope (OR = 2.0; 95%CI: 1.4-2.9), nausea or vomiting (OR = 1.6; 95%CI: 1.1-2.4), and dyspnea (OR = 1.4; 95%CI: 1.1 to 1.9), than in those with painless presentations of unstable angina (OR = 2.2; 95%CI: 1.4-3.5) and ST-segment elevation MI (STEMI) (OR = 1.7; 95%CI: 1.2-2.2). In patients with unstable angina and non-ST elevation MI, 5.7% and 12.3% had atypical symptoms, respectively. In addition, patients with atypical presentation had less coronary angiography and subsequent revascularization, anticoagulant, antiplatelet and B-blocker therapy. These patients were also less likely to receive aspirin, B-blockers, or statins after discharge, this was seemingly linked to the failure to identify the diagnosis initially. Bearing in mind the higher baseline risk of

the population presenting without chest pain, those with atypical presentation frequently had in-hospital complications. On the other hand, the excessive mortality rate seen in the GRACE study was marked with almost 20% in-hospital mortality in the silent STEMI patients. Nevertheless, the absence of chest pain resulted in a greater probability of in-hospital death in all patients with ACS, and, even after multivariate analysis, the excessive mortality rate persisted among patients with unstable angina and STEMI^[76].

Studies which did not show that diabetes mellitus is a predictor of atypical presentation of acute coronary syndrome (Table 3)

Numerous studies^[77-81] have shown that diabetes mellitus is not a predictor of atypical presentation of ischemic syndrome. A study examined the disparities between Mexican Americans and non-Hispanic whites in the described symptoms of AMI. The symptoms in patients in a community-based surveillance program were determined to establish the differences between groups in relation to ethnicity, gender, and diabetic status. Information concerning the symptoms of 589 patients hospitalized and identified as having either definite or possible AMI (aged 25 to 74 years) was obtained. Chest pain was the most frequent complaint (83.2%), followed by chest

pressure or discomfort (67.6%), sweating (64.2%), fatigue (62.6%), dyspnea (60.3%), and arm or jaw pain (58.2%). After adjusting for age, diabetes mellitus, and gender, and relative to non-Hispanic whites, Mexican Americans frequently reported chest pain, upper back pain, and palpitations, but were less likely to report arm or jaw pain. Similarly, women predominantly reported fatigue, dyspnea, dizziness, upper back pain, palpitations, and cough, but less frequently reported chest pain. Substantial differences were observed in older compared to younger patients' symptoms^[77].

Diabetics with AMI may present similar to non-diabetics. In a prospective, observational study in patients with typical and atypical symptoms consistent with cardiac ischemia, 216 diabetic and non-diabetic patients with AMI were compared, 24% were diabetic, with no significant difference in age ($P = 0.13$), female gender ($P = 0.13$), and time to presentation from symptom onset (192 ± 238 min *vs* 251 ± 456 min, $P = 0.41$). For diabetic *vs* non-diabetic with AMI, hypertension was more common in diabetic compared with non-diabetic patients with AMI (77% *vs* 50%, $P = 0.001$), and the same applied to elevated cholesterol (48% *vs* 33%, $P = 0.06$). No significant differences between diabetics and non-diabetics in terms of the frequency of chest pain (OR = 1.04; 95%CI: 0.95-1.14, $P = 0.30$), associated symptoms, and diagnostic ECGs (OR = 1.16; 95%CI: 0.76-1.79, $P = 0.53$) were observed^[78].

Data from 2 registries of AMI patients presenting in hospital (MITRA PLUS with 18786 patients; North German Registry, NGR), analyzed AMI symptoms in 1042 diabetic and non-diabetic patients. Diabetics were significantly older and more often female than non-diabetics. No difference in the incidence of pre-infarction angina between the 2 groups (Mitra Plus) was observed. In the NGR, severe angina during AMI was perceived in 49.8% of diabetics *vs* 46.3% of non-diabetics ($P = \text{NS}$). In addition, 16.9% of diabetics and 15.0% of non-diabetics (P ; NS) had SMI with no disparity in extra-thoracic pain, dizziness, nausea, sweating, palpitations, radiation of angina and localization of radiating pain in diabetics *vs* non-diabetics. Severe dyspnea occurred in 29.5% of diabetics and 19.5% of non-diabetics patients ($P = 0.003$). In this analysis, apart from a higher frequency of severe dyspnea in diabetics, no differences in the clinical symptoms of AMI patients with and without diabetes mellitus were noted. Silent or minimally symptomatic AMI was more common in non-diabetics^[79]. A study determined the differences in symptoms in patients (50 women and 50 men) with and without diabetes during an episode of unstable angina. In this study diabetics were more frequently hypercholesterolemic (83% *vs* 60%), had a past cardiac history (85% *vs* 65%), and prior angiogram (85% *vs* 67%). Diabetics had less nausea (20% *vs* 40%), less squeezing (25% *vs* 48%) and less aching (25% *vs* 45%) pain, with more frequent hyperventilation as the presenting symptoms (27.5% *vs* 11.7%). With no difference in other cardiac symptoms seen in the two groups^[80].

SILENT AND ATYPICAL MYOCARDIAL ISCHEMIA IN DIABETICS: TO SCREEN OR NOT?

Assuming a greater risk of cardiovascular events and more frequent silent CAD in diabetics compared to non-diabetics, screening asymptomatic diabetic patients for CAD is an attractive concept. Nevertheless, there are many elements against instigating a wide-ranging screening program. Of note is the paucity of confirmed data indicating that a prospectively utilized screening program has a positive prognostic impact in asymptomatic diabetic patients. From the above reviewed studies the incidence of atypical SMI is highly variable. Measures should be taken to manage hypertension and hyperlipidemia exclusively on the basis of diabetes status, devoid of diversity based on the presence or absence of recognizable CAD. From the above available data the studies which used stress single-photon emission computed tomography imaging showed around 50% abnormal images and 20% high-risk images, respectively. However, the DIAD (Detection of Ischemia in Asymptomatic Diabetics) study^[42] described a considerably lower percentage of abnormal SPECT images (16%) and images with a very large ($\geq 10\%$ of the left ventricle) defect of 1%. We think that it is wise for the clinician to investigate silent and/or atypical myocardial ischemia and this applies to stable CAD in high risk diabetic patients, *i.e.*, patients with long-standing diabetes and diabetic complications such as diabetic neuropathy which may frequently present atypically. We suggest using a test which has high specificity and sensitivity for the detection of myocardial ischemia such as a myocardial perfusion scan and SPECT scan as shown in the above studies. The massive fiscal consequences of investigating all asymptomatic diabetic patients at intermediate and high risk using clinical scoring systems should be considered. Undoubtedly more investigations are required to address these issues.

CONCLUSION

Not all diabetics have the same coronary risk, therefore, it is important to determine which investigations to perform and for which patients. This strategy is reasonable as it allows identification of patients who require a medical or an invasive (angioplasty *vs* CABG) procedure, as these interventions may improve the prognosis. Patients with more than two risk factors may need further investigations with exercise stress testing which may provide supporting diagnostic and prognostic data. When exercise stress testing is sub-maximal or non-diagnostic, a second investigation with perfusion myocardial scintigraphy may be warranted bearing in mind that in diabetics this test may not have the same diagnostic accuracy as in the general population, but it is of prognostic value. Ischemia involving over 20%-25% of the myocardium justifies therapeutic investigation. Stress echocardiography is

comparable to scintigraphy.

The greater incidence of SMI in diabetics seems to be due to the increased frequency of ischemic heart disease in diabetics. The importance of cardiac autonomic neuropathy in SMI is still debatable, but is the most acceptable cause of SMI, as discussed in the above review, nevertheless studies are sporadic. The risk factors associated with SMI and atypical ischemic syndrome are the usual traditional factors *i.e.*, age, male gender, hypercholesterolemia, hypertriglyceridemia, hypertension, smoking, a family history of cardiovascular disease, insulin therapy (for type II diabetes), proteinuria, retinopathy, and peripheral occlusive arterial disease. Upcoming studies should determine possible approaches to augment the patient subgroup that will possibly benefit from screening with judicious cost-effective analyses. Currently, there are no data to support the use of anti-ischemic medication to improve CAD in diabetic patients.

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Renal sympathetic nervous system and the effects of denervation on renal arteries

Arun Kannan, Raul Ivan Medina, Nagapradeep Nagajothi, Saravanan Balamuthusamy

Arun Kannan, Saravanan Balamuthusamy, Department of Medicine, University of Arizona, Tucson, AZ 85721, United States

Raul Ivan Medina, Saravanan Balamuthusamy, Vascular and Interventional Nephrology, Angiocare, Tucson, AZ 85721, United States

Nagapradeep Nagajothi, Cardiovascular Consultants, Canton, OH 44710, United States

Author contributions: Kannan A and Balamuthusamy S made substantial contributions to conception and design, acquisition of data; Kannan A, Medina RI and Nagajothi N contributed by drafting the article and revising it critically for important intellectual content; and Balamuthusamy S contributed to the final approval of the version to be published.

Correspondence to: Saravanan Balamuthusamy, MD, FASN, Assistant Professor, Director of Vascular and Interventional Nephrology, Angiocare, 224 W Exchange St, Tucson, AZ 85721, United States. sbalamuthusamy@email.arizona.edu
Telephone: +1-520-3276265 Fax: +1-520-3279300

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Abstract

Resistant hypertension is associated with chronic activation of the sympathetic nervous system resulting in various comorbidities. The prevalence of resistant hypertension is often underestimated due to various reasons. Activation of sympathetic nervous system at the renal- as well as systemic- level contributes to the increased level of catecholamines and resulting increase in the blood pressure. This increased activity was demonstrated by increased muscle sympathetic nerve activity and renal and total body noradrenaline spillover. Apart from the hypertension, it is hypothesized to be associated with insulin resistance, congestive heart failure and obstructive sleep apnea. Renal denervation is a novel procedure where the sympathetic afferent and efferent activity is reduced by various techniques and has been used successfully to treat drug-resistant hypertension improvement of various metabolic derangements.

Renal denervation has the unique advantage of offering the denervation at the renal level, thus mitigating the systemic side effects. Renal denervation can be done by various techniques including radiofrequency ablation, ultrasound guided ablation and chemical ablation. Various trials evaluated the role of renal denervation in the management of resistant hypertension and have found promising results. More studies are underway to evaluate the role of renal denervation in patients presenting with resistant hypertension in different scenarios. Appropriate patient selection might be the key in determining the effectiveness of the procedure.

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Key words: Resistant Hypertension; Sympathetic nervous system; Sympathectomy; Renal denervation; Radiofrequency ablation

Core tip: Resistant Hypertension is a serious condition that could result in various comorbidities, if left untreated. The pathogenesis involves activation of sympathetic nervous system at the renal level and systemic level. Surgical therapy targeted at the systemic level has serious systemic side effects. Renal denervation offers a unique way of mitigating the chronic activation of sympathetic nervous system and controlling the high blood pressure.

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INTRODUCTION

American Heart Association^[1] and Joint National Committee^[2] define resistant hypertension as blood pressure

that remains uncontrolled with the patient remaining compliant to 3 or more drugs, one of them being a diuretic. Care should be taken to differentiate resistant hypertension from uncontrolled hypertension, as the latter may be due to sub-optimal therapy, non-adherence to medications and secondary hypertension. The prevalence of resistant hypertension is often under estimated due to various reasons including inadequate sample size, exclusion of patients with resistant hypertension in larger studies^[3,4]. Kaplan *et al*^[3] have estimated that up to 5% of patients in general medicine clinics and approximately 50% of patients seen in renal clinics have resistant hypertension.

An important consideration in defining a patient with resistant hypertension is the frequent mislabeling of secondary hypertension as resistant hypertension and not addressing the issue of non-adherence to optimal therapy. This has been frequently reported in the literature including white-coat hypertension^[5], non-compliance^[6], secondary hypertension^[1], and isolated systolic hypertension^[7].

ROLE OF SYMPATHETIC NERVOUS SYSTEM IN HYPERTENSION

Renal sympathetic efferent and afferent nerves, which lie adjacent to the wall of the renal artery, are crucial for production of catecholamines contributing to hypertension. Surgical sympathectomy, targeted at removing sympathetic ganglia, to control hypertension has been reported even before the advent of newer antihypertensives^[8]. Due to its profound side effects and the introduction of pharmaceutical sympatholytic agents, surgical sympathectomy is not a preferred procedure anymore. Renal denervation is a novel technique, which involves selective ablation of renal sympathetic nerve fibers and has demonstrated promising results in controlling resistant hypertension. The renal nerves are sensitive to ablation techniques such as radiofrequency and ultrasound.

RENAL SYMPATHETIC DENERVATION AND HYPERTENSION

Various types of primary and secondary hypertension, including essential hypertension^[9], renovascular hypertension^[10], hypertension associated with disordered sleep breathing^[11], hypertension associated with Cushing's syndrome^[12] and primary aldosteronism, and preeclampsia, have been shown to have an association with sympathetic nervous system in various human and animal models.

Initially postulated to control circulation, sympathetic nervous system has been found to play a crucial role in initiation and maintenance of systemic hypertension through its effects on renal blood flow and perfusion^[13,14].

Renal sympathetic nervous system consists of afferent and efferent sympathetic nerve fibers adjacent to the adventitious layer of the renal arteries. Efferent sympathetic nerves, when stimulated, have multitude of effects including increased renin secretion, decreased renal blood flow and increased renal tubular sodium absorption^[15].

These changes contribute to the increased fluid retention and sustenance of vascular hypertension. Such sympathetic nerve fiber stimulation also contributes to increased renin mediated Angiotensin-Aldosterone activity further augmenting the hypertension.

The physiological effects of sympathetic nervous system in initiation, and maintenance of blood pressure makes it an excellent therapeutic target for drug and procedure based intervention in the management of hypertension. In animal models, Roman *et al*^[16] has demonstrated that denervation resulted in leftward shift in the pressure natriuresis curve implying increased excretion of both water and sodium with no change in renal perfusion pressure.

Various types of hypertension have been shown to be ameliorated by renal denervation in different experimental models^[14]. These experiments explored the role of efferent sympathetic nerve fibers in pathophysiology of development and maintenance of hypertension. Renal afferent sensory fibers act through a different mechanism in maintaining sodium and water homeostasis. These fibers are found primarily in the renal pelvic wall and they act through substance P and calcitonin gene related peptide, both of which act as primary neurotransmitters^[17]. These fibers, by responding to changes in pressure in renal pelvis (mechanoreceptors) and chemical characteristics (chemo sensitive receptors) of urine, increase diuresis and natriuresis^[18].

Activation of the efferent renal sympathetic nerve fibers can occur in response to augmented afferent signaling from renal sensory nerve fibers caused by various stimuli such as renal ischemia, hypoxia, and oxidative stress^[19,20].

Renal afferent nerve fibers send signals to the hypothalamus and stimulate sympathetic outflow, causing hypertension and increased systemic vascular resistance^[21,22]. Hausberg *et al*^[23] reported similar effects of increased activity of sympathetic outflow due to renal afferent nerve signaling in end stage renal disease. In a recent study, Ceral *et al*^[24] measured the serum drug levels of prescribed antihypertensive drugs to evaluate the adherence in individuals with difficult-to-control hypertension. In 65% patients, non-adherence was diagnosed. In upto 34 patients, no drugs were detected underscoring the importance of recognizing non-adherence in this population.

Such effects are also observed in patients with chronic kidney disease including end stage renal disease. Significant decreases in sympathetic activity have been demonstrated in patients with bilateral nephrectomy^[25]. Converse^[26] recorded the rate of sympathetic-nerve discharge to the muscular blood vessels in patients with chronic kidney disease with and without renal transplantation. He reported significant sympathetic over activity in End Stage Renal Disease (ESRD) patients with and without renal transplantation compared to normal subjects and in ESRD patients who had undergone nephrectomies. It has also been demonstrated that there is upto 30% sympathetic nerve re-innervation even in transplanted kidneys.

Further evidence of association of sympathetic over

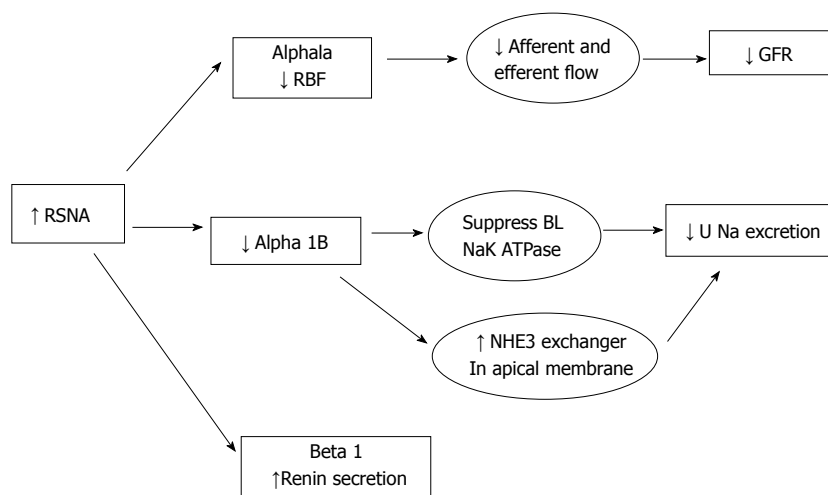


Figure 1 Showing intra-renal mechanisms of renal sympathetic activity. RSNA: Renal sympathetic nerve activity; RBF: Renal blood flow; GFR: Glomerular filtration rate; NHE: Sodium hydrogen transporter; U Na: Urinary sodium; BL: Baso-Lateral membrane.

activity with hypertension was seen in patients with obstructive sleep apnea. Marshall^[27] and Cooper *et al*^[28] elucidated that hypoxia seen in OSA results in increased sympathetic outflow to renal, cardiac and splanchnic beds and associated hypertension.

INTRA-RENAL FUNCTIONS OF THE RENAL SYMPATHETIC NERVOUS SYSTEM

It is elucidated that various physiological aspects of kidneys are regulated by sympathetic nervous system. Activation of sympathetic nerve fibers at the renal level results in locally increased release of norepinephrine and renin. This leads to renal vasoconstriction, decreased renal blood flow resulting in decreased glomerular filtration rate and increased renal tubular reabsorption of sodium and water at the tubular level^[13]. Figure 1 shows intra-renal mechanisms of renal sympathetic activity

It is important to understand the physiological effects of sympathetic nervous system on the different ultra structural components in the kidneys to be aware of the outcomes of sympathetic denervation.

Renal blood flow: there is a decrease in renal blood flow with increased renal sympathetic nervous system activity. This decrease in flow is primarily mediated through increased afferent renal arteriolar vasoconstriction. There is also efferent arteriolar constriction, which helps sustain effective filtration pressure to sustain glomerular filtration rate (GFR).

Renal tubules

There is extensive sympathetic innervation in the entire renal tubule. The innervations are most dense in the thick ascending loop of Henle (TALH) followed by the proximal tubule, distal tubule and the cortical collecting duct. Activation of the SNS suppresses the Na^+K^+ ATPase at the basolateral membrane, which provides the energy for most of the transcellular transport that occurs across the luminal side of the tubules. There is also increased activation and expression of the NHE3 exchanger in the apical

membrane which leads to increased Na retention across the tubules. The NKCC2 transporter at the TALH is also activated with SNS activation, which enhances salt absorption at this segment further increasing salt retention.

Renin secretion

Activation of the ERSNS increases rennin mRNA and therefore increases plasma and renal renin secretion. The increased renin secretion is partially mediated through the effects on the baroreceptors at the afferent renal arterioles. This increased renin secretion happens at low renal perfusion pressure even with minimal sympathetic nervous activation. The baroreceptor mediated renin release does not occur at high renal perfusion pressure states.

Reno-renal reflex

Increased pelvic pressure or high salt intake activates ARSN and thereby inhibits the ERSNA hence decreases salt retention and decreases blood pressure in normal kidneys. However in ischemic kidneys or chronic hypertension there is a reversal of the reno-renal reflex and ARSN activity further enhances the sympatho excitatory state and increases salt retention and hypertension. This reversal of the reno-renal reflex is significant since there is a greater expression of the afferent sympathetic nerves in patients with hypertension when compared to normotensive controls.

The above mechanisms enunciate the intricate significance of SNS and renal physiology in the development and maintenance of hypertension.

The increased sympathetic fiber traffic can be measured by microneurography- a clinical method of measuring multi^[29] - and single^[30] fiber activity in skeletal muscle fibers in humans. The measurement of microneurography allows direct and accurate measurement of NE activity when compared to measurement of plasma catecholamines.

Organ specific (for example, cardiac and renal) norepinephrine release can be quantified by "Norepinephrine Spillover" technique, which involves measuring organ specific outward flux of endogenous norepinephrine^[31].

Although, renovascular hypertension and hyperten-

Table 1 Different techniques of renal denervation

Approach	Technique	Device	Study	Follow-up	Outcome
Invasive	RF ablation	Balloon: OneShot	Renal hypertension ablation system trial ^[69]	12 mo	Average reduction in BP = 30.6 ± 22.0
		Vessix	REDUCE-HTN	Ongoing	
		Non-balloon: Simplicity	SIMPLICITY I ^[35] SIMPLICITY II ^[62] SIMPLICITY III	24 mo 6 mo Ongoing trial	32/14 32/12
	Ultrasound	Spiral	Renal hypertension ablation system trial ^[69]	12 mo	Average reduction in BP = 30.6 ± 22.0
		EnligHTN	EnligHTN I trial ^[70]	6 mo	Reduction in BP-26/10
		Paradise	REALISE ^[71]	3 mo	Reduction in BP 22/12
Non-invasive	Ultrasound	TIVUS	TIVUS I	Ongoing study	
		Verve			
	Chemical	Cisplatin	Salman ^[72]	Animal study	
		Vincristine	Silva	Animal study	
		Guanethidine	Koistinaho ^[73]	Animal study	
	Other	Neurotoxin	Apex nano nanomagnetic therapy	Animal studies	
		Beta radiation cath	Novoste ^[74]		

sion due to chronic kidney disease are separate clinical entities than essential hypertension, they somehow share a common pathway with enhanced sympathetic nervous activity and activation of Renin Angiotensin Aldosterone System.

RENAL DENERVATION

Several experimental models have explored the role of renal sympathetic efferent and sensory afferent nerves in systemic and renal function by renal denervation. These experiments were done by surgical ligation and by surgical ablation of the renal nerve with phenol application in the adventitia of the renal arteries. The role of renal denervation was explored in clinically significant medical conditions such as hypertension^[32], chronic kidney insufficiency^[25] and in chronic heart failure in the past decades^[33]. Dibona^[13] elucidated the role of bilateral renal denervation in decreasing the sympathetic nerve fiber activity in various animal models including reno-vascular hypertension and chronic renal failure to reducing hypertension.

Renal denervation not only reduces renal sympathetic efferent activity selectively, but also decreases in whole body efferent sympathetic activity. Schlaich *et al.*^[34] and Krum *et al.*^[35] reported a considerable reduction in renal nor adrenaline spillover and a reduction in plasma renin activity^[34]. Renal denervation also has shown to reduce whole-body noradrenaline spillover, evident by reduced sympathetic nerve signaling to the skeletal muscle vasculature. In a recent study, Hering *et al.*^[36] found substantial and rapid reduction in firing properties of single and multiple sympathetic vasoconstrictor nerve fibers.

The role of sympathetic nervous system in renovascular hypertension is well studied in animal models^[37, 38]. Although, no study has been done in human models to evaluate the role of renal denervation in renovascular hypertension, the critical association of SNS activity and re-

sistant hypertension is well established^[38]. Table 1 shows different techniques of renal denervation.

SURGICAL SYMPATHECTOMY

As previously mentioned, sympathectomy was considered an effective modality of controlling hypertension as early as 1930s^[39]. Splanchnicectomy, which includes sympathectomy of abdominal organs, was poorly tolerated due to its significant side effects including orthostatic hypotension, palpitations, anhidrosis and ejaculation defects^[40]. Later, more conservative surgeries were performed at the level of thoracic vertebra^[41]. Although a satisfactory blood pressure control and improvement of survival was seen in almost 50% of patients, it was not widely performed due to its adverse systemic effects. Advent of novel anti hypertensive medications has shifted the focus towards drug therapy in controlling severe hypertension. Sympathectomy has been reserved for severe resistant hypertension not responsive to medications.

CLINICAL STUDIES ON RENAL DENERVATION

Renal denervation offers the advantage of sympathectomy, yet involves denervation at the renal level largely avoiding the adverse effects of sympathectomy. Table 2 shows different clinical trials on renal denervation.

We present the human data that has demonstrated favorable reduction in blood pressure after renal denervation.

In SIMPLICITY I trial, 45 patients were included and radiofrequency ablation was done using a treatment catheter (Simplicity by Ardian Inc, Palo Alto, CA, United States). This is a non-randomized, prospective proof of concept study. Patients were eligible if they had systolic blood pressure of 160 mmHg or more, despite optimal

Table 2 Different clinical trials on renal denervation

Trial	Mean followup	Reduction in SBP/DBP	Location	Type	Primary outcome	Safety data
SIMPLICITY I 2009, (n = 50) ^[38]	6 mo	22/11	Australia/Europe	Catheter-based	Substantial and sustained BP reduction w/o serious adverse events	One case of Renal artery dissection
	12 mo	27/17			Substantial and sustained BP reduction w/o serious adverse events	
SIMPLICITY I F/u study 2011 ^[42] (n = 153)	24 mo	32/14	Australia/Europe/United States	Catheter-based	Substantial BP reduction	Groin pseudoaneurysms
SIMPLICITY II 2010, (n = 106) ^[43]	6 mo	32/12	Australia/Europe/United States	Catheter-based	Meaningful reduction in BP	Hypertensive emergency in 3 cases
Mahfoud 2013, (n = 245) ^[48]	3 mo	19/13				
(n = 236)	6 mo	17/12				
(n = 90)	12 mo	16/10	Australia/Germany	Catheter-based	RDN improved BP relevantly in office and ambulatory scenarios	No adverse events reported
Witowski <i>et al</i> ^[49]	6 mo	34/13	Poland/United States	Catheter-based	Improvement in severity of sleep apnea, glucose tolerance and BP	No adverse events reported
Brandt <i>et al</i> ^[75]	6 mo	29/8	Austria/Germany	Catheter-based	Improves BP, arterial stiffness and central hemodynamics	No adverse events reported
2012 (n = 110)						
Davies <i>et al</i> ^[76] 2012, (n = 7)	6 mo	7/0.6	United Kingdom/United States	Catheter-based	Improvement in symptoms and exercise capacity	No adverse events reported
Esler <i>et al</i> ^[62] 2012 (n = 106)	24 mo	32/12	Australia/Europe/United States	Catheter-based	Safety and continues benefit with denervation	Hypotension after denervation
Hering <i>et al</i> ^[64] 2012 (n = 15)	6 mo	32/15	Australia/Europe/United States	Catheter-based	Safe and BP beneficial in resistant HTN and CKD stage 3-4	No peri- or postprocedural complications reported
	12 mo	33/19			Safe and BP beneficial in resistant HTN and CKD stage 3-4	
Mahfoud <i>et al</i> ^[77] 2011	3 mo	28/10	Germany	Catheter-based	Reduction in BP and glycemic control	None reported
Lambert <i>et al</i> ^[78] 2012, (n = 40)	3 mo	16/6	Australia/Europe	Catheter-based	Quality of life improved after denervation but not directly associated to BP reduction	None reported
Mahfoud <i>et al</i> ^[77] 2011, (n = 37)	1 mo	28/10				
	3 mo	32/12	Australia/Germany	Catheter-based	Improvement in glucose levels and insulin sensitivity in addition to BP reduction	No significant adverse events reported
Ott <i>et al</i> ^[63] 2013, (n = 19)	6 mo	16/7	Germany/United States	Catheter-based	Significantly improvement in peripheral and central BP	No changes in renal function and perfusion
Schlaich <i>et al</i> ^[61] 2013, (n = 9)	3 mo	18/4	Germany/Australia/Poland/United States	Catheter-based	RDN causes sustained lower BP in ESRD	One patient developed femoral pseudo-aneurysm
(n = 8)	6 mo	16/6				
(n = 6)	12 mo	28/5				
Steinberg <i>et al</i> ^[51] 2013, (n = 13)	12 mo	25/10	United States	Catheter-based	RDN patients displayed a significant reduction in systolic and diastolic pressure and maintained	No Adverse events reported
Ukena <i>et al</i> ^[50] 2012, (n = 2)	6 mo	No Info	Germany/ United States	Catheter-based	Ventricular tachyarrhythmias significantly improved after RDN	No complications reported
Vaclavik <i>et al</i> ^[79] 2013, (n = 1)	3 mo	No effect in this unilateral procedure	Czech Republic	Catheter-based	Unilateral Renal sympathetic denervation does not lower BP	No complications reported

CKD: Chronic kidney disease.

therapy with three antihypertensive drugs or more (including a diuretic). The primary endpoint was safety and reduction in blood pressure after the procedure and secondary endpoints were effects of the procedure on renal noradrenaline spillover and renal function. Patients with secondary hypertension including reno-vascular hypertension were excluded. The follow-up period was 1 year. There was a significant reduction in systolic and diastolic

blood pressure at 1- and 3-mo follow up which remained consistent through out the follow-up period. In this proof of principle study, they found that the reduction in blood pressure was consistent suggesting neither significant nerve fiber recovery nor the development of any counter-regulatory mechanisms. Six patients did not have any response to treatment suggesting a possible different mechanism in the development of resistant hypertension

or inadequate therapeutic renal denervation.

The same study^[42] was continued for a total of 24 mo and a sustained reduction in blood pressure was seen in the cohort.

SIMPLICITY II^[43] trial is a multicenter, prospective, randomized trial, which compared 52 patients who underwent renal denervation with 54 controls. During the follow-up of six months, patients with renal denervation had a decrease of blood pressure upto 32/12 mm HG, whereas control group had no reduction in blood pressure. This study included 11 patients with early stage 3 CKD (GFR 45-60 mL/min per square meter) and found no significant worsening of renal function. Ambulatory blood pressure monitored in 20 patients in the study population showed a reduction of 11/7-mmHg.

Brinkmann *et al*^[44] analyzed a small subset of patients ($n = 12$) who underwent renal denervation. Mean follow up period was 6 mo. Only 3 patients had clinically significant reduction in blood pressure ($157 \pm 7/85 \pm 4$ mmHg before and $157 \pm 6/85 \pm 4$ mmHg after renal denervation). No significant reductions in sympathetic nerve fiber activity were noted either [prior to denervation- 34 ± 2 bursts per minute and after denervation -32 ± 3 bursts per minute ($P = 0.6$)]. In 7 patients, post denervation blood pressure was actually higher compared to the pre denervation blood pressure. Interestingly, 5 out of 12 patients did not meet the criteria for resistant hypertension (pre denervation BP was less than 140/90).

In a recent study done on patients with resistant hypertension sent for renal denervation workup, Fadl Elmula *et al*^[45] reported only 6 of 18 patients met criteria for resistant hypertension that underwent renal denervation. Twelve patients did not meet the criteria of resistant hypertension for different reasons, including one patient with primary hyperaldosteronism, one with renal artery abnormality and, five patients with normalized ambulatory blood pressure after witnessed drug intake. Out of those 6 patients, only 2 had decreased blood pressure that was sustained for 6 mo.

In a recent report, Vonend *et al*^[46] reported a brief reduction in blood pressure followed by resurgence of hypertension and occurrence of renal artery stenosis after the renal denervation.

Savard *et al*^[47] reported that only a fraction of patients with resistant hypertension referred for renal denervation actually qualified for denervation based on the strict guidelines laid out by European Society of Hypertension.

Mahfoud *et al*^[48] reported the results observed in 303 resistant hypertensive patients and followed them for a period of 12 mo. At 3, 6, and 12 mo follow-up, office systolic blood pressure (SBP) was reduced by 21.5/23.7/27.3 mmHg, office diastolic blood pressure (BP) by 8.9/9.5/11.7 mmHg, and pulse pressure by 13.4/14.2/14.9 mmHg ($n = 245/236/90$; P for all < 0.001). Response to RDN has been defined as a reduction in office SBP ≥ 10 mmHg 6 mo after treatment.

The most recently concluded SIMPLICITY 3 trial in North America has demonstrated that the primary outcomes were not met on initial analysis of the results.

However the study has demonstrated safety in the patients who underwent the denervation procedure. The study was a randomized single blinded case controlled study, which included a sham procedure on the control arm. The primary outcome was decrease in office blood pressure and the secondary outcome was reduction in ambulatory blood pressure at the end of the 6 mo follow-up. Despite the failure of this trial to demonstrate a significant reduction in blood pressure when compared to a sham procedure, an inference cannot be drawn that renal artery denervation is not an effective therapeutic modality anymore. The efficacy of the denervation with this catheter has not been compared to other devices, which use other modalities of generating energy (ultrasound, laser, *etc.*) to ablate the renal nerves. Also the magnitude of denervation has not been assessed in the SIMPLICITY 3 trial. This raises the question if the denervation achieved in the SIMPLICITY 3 trial was adequate to achieve the clinical benefits seen in some of the European and Australian studies. Further analysis from this study would enlighten most of us with the reasons for the lack of benefit from renal artery denervation in this well performed study.

RENAL DENERVATION IN OTHER CONDITIONS

Obstructive sleep apnea

Witkowski *et al*^[49] studied 10 patients with refractory hypertension and sleep apnea who underwent renal denervation and were evaluated at 3- and 6-mo after the procedure. Changes in ambulatory blood pressure and polysomnography were monitored during the follow-up period. Three and 6 mo after the denervation, decreases in median office systolic and diastolic BPs were: -34/-13 mmHg at 6 mo. In addition to the reduction in blood pressure, there was also an improvement in glycemic control and decrease in apnea-hypopnea index.

Arrhythmias

Ukena *et al*^[50] first reported the role of renal denervation in successfully treating two patients with refractory ventricular tachycardia storm.

In a recent study, Steinberg *et al*^[51] enrolled 27 patients with atrial fibrillation (14 randomized to Pulmonary Vein Isolation alone and 13 randomized to Pulmonary Vein Isolation with Renal denervation). The follow-up period was 12 mo after ablation. At 12 mo, the reductions in systolic and diastolic blood pressures were successfully and significantly maintained ($P < 0.001$ vs pulmonary vein isolation only) resulting in a fall from baseline of 25 ± 5 mmHg and 10 ± 2 mmHg, respectively. This effect was thought to be due to increased atrial stretching and dilation (*i.e.*, atrial substrate), when blood pressure is elevated resulting in deleterious atrial electrical consequences that promote AF. With the ablation of afferent renal nervous input, central sympathetic output is decreased and autonomic triggers and substrate potentiators of AF are at-

tenuated.

The results were similar to another study^[52] that demonstrated a decreased incidence of AF recurrences in patients that underwent both pulmonary vein isolation (PVI) and renal artery ablation over time compared with the control PVI-only group.

Chronic kidney disease

Renal denervation has been studied in chronic kidney disease (CKD) - another subset of patients known to have resistant hypertension. Although the decreased clearance of catecholamines was thought to be a factor, the theory was not proven by enhanced clearance upon postural changes^[53]. Levitan *et al*^[54] demonstrated that clonidine (acting as a sympatholytic) significantly decreases norepinephrine secretion and mean blood pressure when compared to controls in patients with chronic kidney disease. Various mechanisms including increased catecholamine sensitivity^[55], renal ischemia^[56] and decreased oxygen supply^[57] have been proposed as the reason for refractory hypertension in CKD patients. Nevertheless, sympathetic hyperactivity is prevalent in CKD and its role in organ damage is well substantiated.

Zoccali *et al*^[10] demonstrated that sympathetic over activity in ESRD is an independent predictor of fatal and non-fatal cardiac events. Hering *et al*^[58], had performed bilateral renal denervation in 15 patients with resistant hypertension and stage 3-4 CKD (mean GFR of 31 mL/min per 1.73 m²) and found consistent reduction in blood pressure with an average systolic and diastolic blood pressure decrease of 34 mmHg and 19 mmHg respectively. They also reported considerable reduction in nocturnal BP control. This is of much importance as nocturnal BP has been shown to predict cardiovascular mortality in hypertensive patients^[59,60].

Hering *et al*^[58] performed bilateral renal denervation in 15 patients with resistant hypertension and stage 3-4 CKD. Mean changes in office systolic and diastolic BP at 1, 3, 6, and 12 mo were -34/-14, -25/-11, -32/-15, and -33/-19 mmHg, respectively.

In another study, renal denervation was performed in 12 ESRD patients^[61] and significant reduction in blood pressure was seen in all subjects.

Few other studies^[62,63] also found similar effects in patients with chronic renal disease.

Insulin resistance and obesity

In a recent study, Hering *et al*^[64] found reduction in fasting glucose and insulin levels in patients treated for resistant hypertension by renal denervation.

Obesity related hypertension has been associated with resistant hypertension^[65]. Holecik *et al*^[66] surveyed approximately 5000 patients with obesity and hypertension and found an increased need in the number of anti-hypertensive medications used with an increase in BMI. Approximately 12% of patients with body mass index (BMI) between 30 and 34.9, 16% of patients with BMI between 35 and 39.9 and 26% of patients with BMI > 40% were found to have resistant hypertension. Although the abso-

lute pathophysiology of hypertension in obese patients has not been well elucidated, experimental and clinical studies conducted in the past few decades have demonstrated an association between increased sympathetic activity and obesity. This association was demonstrated by Rumantir^[67], who found twice normal increase in mean renal noradrenaline spillover in normotensive as well as hypertensive obese patients compared to non-obese patients. The effect of renal denervation in reducing the blood pressure was studied in obese dogs with hypertension^[68]. Renal denervation decreased plasma renin activity and abolished the hypertension in those dogs but failed to suppress systemic sympathetic activity.

COST-EFFECTIVENESS

Most of the renal denervation procedures are performed in Europe, as the device for catheter-based denervation has not been approved by FDA for clinical use in the United States. The average cost of the catheter is 2000 to 3000 United States dollars. The average cost for the procedure in Germany is 4000 to 5000 United States dollars. Patients are admitted to the hospital and observed overnight after the procedure. All the studies published so far have demonstrated blood pressure lowering affects few days to months after the procedure, there have not been reports of a precipitous immediate reduction in blood pressure after the procedure. Hence the reason for an overnight stay is difficult to justify for a procedure that is similar to most catheter based procedures performed through a femoral arteriotomy. Over 60 million population in United States are estimated to have hypertension. Cost and appropriate patient selection would be a major determinant next to patient outcomes in implementing denervation program for hypertensive patients in the United States. Practices with certified hypertension specialists and a suitable arrangement for performing these procedures as an outpatient would be ideal for appropriate patient selection and reducing cost.

CONCLUSION

Renal nerve denervation has been explored as a modality of treatment for resistant hypertension for several decades. Targeting renal nerves through a non-surgical approach has generated more interest in pursuing denervation as an option for hypertension refractory to conventional medical management. Despite controversies in the true prevalence of resistant hypertension, the existence of such a disease is beyond clinical doubt. Long-term patient outcomes including mortality and renal outcomes are yet to be substantiated with evidence from on-going and future trials with hard outcomes. Though the SIMPLICITY 3 trial did not reach primary outcomes, there could be other systemic benefits secondary to sympathetic denervation, which is yet to be proven in clinical trials. Also the effectiveness of denervation with the SIMPLICITY catheter has not been compared to other devices capable of denervating the renal arteries. With the established procedural safety from the SIMPLICITY

3 trial it might be safe and cost-effective to perform these procedures in an outpatient setting for a few selected patients who may still benefit from renal nerve denervation.

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Is reversal of endothelial dysfunction still an attractive target in modern cardiology?

Ify Mordi, Nikolaos Tzemos

Ify Mordi, Nikolaos Tzemos, Institute of Cardiovascular and Medical Sciences, University of Glasgow, British Heart Foundation Glasgow Cardiovascular Research Centre, Glasgow G12 8TA, United Kingdom

Author contributions: Mordi I and Tzemos N were sole contributors to this paper.

Correspondence to: Dr. Nikolaos Tzemos, Institute of Cardiovascular and Medical Sciences, University of Glasgow, British Heart Foundation Glasgow Cardiovascular Research Centre, 126 University Place, Glasgow G12 8TA, United Kingdom. niko.tzemos@glasgow.ac.uk

Telephone: +44-141-3302079 Fax: +44-141-3306697

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dependent indicator of adverse prognosis. Despite this, perhaps due to lack of standardisation of investigative techniques, endothelial function assessment is not yet routinely undertaken, despite a number of therapies which have been shown to have beneficial effects on the endothelium. More studies are required to judge whether assessment of endothelial function can impact on clinical management and prognosis.

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Abstract

Although the endothelium has a number of important functions, the term endothelial dysfunction is commonly used to describe impairment in its vasodilatory capacity. There have been numerous studies evaluating the relationship between endothelial dysfunction and cardiovascular disease, however assessment of endothelial function is perhaps still primarily thought of as a research tool and has not reached widespread clinical acceptance. In this review we explore the relationship between endothelial dysfunction and cardiovascular disease, its prognostic significance, methods of pharmacological reversal of endothelial dysfunction, and ask the question, is reversal of endothelial dysfunction still an attractive target in modern cardiology?

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Key words: Endothelium; Vascular; Nitric oxide; Atherosclerosis; Risk factors; Flow-mediated dilatation

Core tip: There is an abundance of evidence suggesting that endothelial dysfunction is present throughout a wide spectrum of cardiovascular disease and is an in-

INTRODUCTION

For many years the vascular endothelium was thought of as simply a selectively permeable barrier between the intra- and extravascular compartments. However, discovery by Furchgott *et al*^[1] that the large blood vessels of mammals only dilate if the endothelium is intact due to its response to nitric oxide (NO) was the first step in our understanding that the endothelium is a key modulator of cardiovascular health. Indeed, the integrity of the vascular endothelium is essential for providing adequate blood flow and antithrombotic activity. While these are key functions of the endothelium, in the context of cardiovascular health, the key function of the endothelium is maintenance of vasodilatation in response to NO. The healthy human endothelium maintains a vasodilated state as a baseline, in part due to NO production from L-arginine by nitric oxide synthase. NO then diffuses into the endothelium, leading to increased cyclic guanosine monophosphate (GMP) production and vasodilatation^[2]. Damage to the endothelium, whether anatomical or functional can cause a disturbance of this pathway leading to endothelial dysfunction. There are three potential mecha-

nisms that can lead to endothelial dysfunction (either in isolation or combination): reduced production of NO^[3], reduced availability of NO^[4] or antagonism of NO by endothelium derived contracting factors^[5]. Indeed, although NO is the main endothelium-derived relaxing factor there are other factors active on the endothelium, all of which play a key role in its health. Other endothelium-derived relaxing factors include prostacyclin and endothelium-derived hyperpolarizing factor, both of which can show increased activity in response to a decrease in NO. Meanwhile, there are several endothelium-derived contracting factors causing vasoconstriction such as endothelin-1, thromboxane A₂ and prostaglandin H₂. Nevertheless, the majority of clinical studies have concentrated on NO, and this will be the focus of our review.

NO has a number of vascular protective roles including inhibition of platelet aggregation and leucocyte adhesion, however endothelial dysfunction can be simply described as the imbalance of vasodilatation and vasoconstriction caused by vasoactive substances acting on the endothelial cells^[6]. Endothelial dysfunction is present in a number of cardiovascular conditions such as diabetes, hypercholesterolemia and hypertension and seems to be an important feature in the pathogenesis of the atherosclerotic disease process.

In this review, we will discuss the association of endothelial dysfunction with the cardiovascular disease, its prognostic relevance, methods for reversing endothelial dysfunction and their impact on outcome.

HOW DO WE QUANTIFY ENDOTHELIAL FUNCTION CLINICALLY?

Theoretically endothelial function can be measured in any artery. In most methods the endogenous NO-dependent vasodilatation is measured using a pharmacological agonist such as acetylcholine (ACh) or other substances which stimulate endogenous NO production. Comparison is then made with NO-independent vasodilatation using substances such as glyceryl trinitrate. Invasive measurement of the coronary artery response to acetylcholine is a validated measurement of coronary artery endothelial function and was the first method used to demonstrate endothelial function^[7,8]. Using quantitative coronary angiography the change in diameter of the artery can be measured in response to ACh. In dysfunctional coronary arteries ACh causes reduced vasodilatation or apparently paradoxical vasoconstriction due to the unopposed direct smooth muscle muscarinic action of ACh at apparently high concentrations^[9].

Non-invasive measures include what is considered by many as gold standard, venous occlusion plethysmography. This technique is used to assess forearm blood flow (in the brachial artery) in response to an inflated blood pressure cuff. The inflation of the cuff occludes venous return (but not arterial inflow) thus creating a “reservoir” of blood within the anatomically isolated limb region (forearm). The rate of vessel swelling can be measured as

a surrogate for vascular resistance while the volume increases in proportionally in relation to the forearm blood flow^[10]. Endothelial function, which is closely related to NO bioactivity, can be measured by constructing dose-response curves to escalating doses of ACh and measuring the rate of change in arm swelling by strain gauge. One advantage of this technique is that measurement of forearm blood flow in the contralateral arm can be used as a further within patient control, allowing optimal reproducibility^[11]. Nevertheless, the requirement for arterial cannulation may limit patient tolerability and repeatability.

Flow-mediated dilation (FMD) is probably the most common method of endothelial function assessment. This technique involves using ultrasound to measure the peripheral arterial response (again, usually the brachial artery) to temporary ischemia caused by inflation and release of a cuff. Release of the cuff causes an increase in blood flow and therefore shear stress which stimulates NO release and leads to vasodilatation. The increase in diameter of the blood vessel from baseline can be measured by two dimensional ultrasound and is related (but not exclusive) to NO bioavailability, giving an excellent measure of endothelial function which can again be compared to dilatation using endothelium-independent vasoactive substances^[12]. Of note, FMD has been shown to have excellent correlation with coronary endothelial function^[13].

A more recently developed method of assessment is peripheral arterial tonometry (PAT). This technique allows non-invasive measurement of vasomotor function by measuring plethysmographic changes in the fingertip pulse. Again, the endothelium-dependent response can be ascertained by arterial cuff occlusion^[14]. PAT has also been shown to correlate well with both coronary endothelial function and FMD^[15,16].

WHAT IS THE CLINICAL RELEVANCE OF ENDOTHELIAL DYSFUNCTION?

While several methods have been developed to assess endothelial function in different arterial beds, there can only be any benefit to quantification of endothelial dysfunction if there is evidence that it can be used to identify groups with an adverse prognosis.

Several studies have shown a relationship between endothelial dysfunction, coronary disease risk factors and atherosclerosis. One of the earliest studies revealing this relationship was carried out by Ludmer *et al*^[8] who discovered that in patients with both mild and advanced coronary artery disease (CAD) there was paradoxical vasoconstriction induced by acetylcholine. Evidence of endothelial dysfunction has also been noted in patients with risk factors for CAD but without angiographically significant CAD, suggesting that endothelial dysfunction may indeed predate the development of clinically significant atherosclerosis^[17,18]. Age^[19], diabetes mellitus^[20-22], smoking (both active and passive)^[23-25], hypertension^[26] and hyperlipidemia^[27,28] have all been associated with endothelial

dysfunction prior to the development of clinically significant CAD. Furthermore, patients with a combination of risk factors (such as smoking and hypercholesterolemia) have been shown to have worse endothelial function than those with a single risk factor^[29].

The presence of endothelial dysfunction has been shown to be a predictor of cardiovascular events independent of the arterial bed studied or method of assessment^[30,31]. Much of this effect is due to the fact that endothelial dysfunction is invariably present whenever there is end-organ damage. This is clinically manifested as atherosclerosis, left ventricular hypertrophy, small vessel brain ischemia and renal impairment, leading to significant morbidity and mortality^[32-34]. Not unreasonably, endothelial function assessment could be considered as the barometer of vascular health^[35]. Large studies investigating the prognostic value of endothelial function assessment using FMD are summarized in Table 1.

So why has endothelial dysfunction assessment not been adopted more widely clinically? As we have discussed, FMD appears to be the most robust and widely used technique, yet it very rarely appears in any clinical guidelines. One reason may be that although FMD does have predictive value, there are of course several other risk factors that may be easier to assess which are also predictors of adverse cardiac outcome^[31]. Secondly, although many studies have reported the excellent reproducibility and variability of FMD measurement in multiple institutions^[36-39], these studies all rely on following an “ideal” protocol for obtaining FMD measurements. According to a recent paper published by the European Society of Cardiology, this includes 10 min rest for the patient prior to measurement, correct cuff placement, an occlusion time of 5 min and measurement 45-60 s after cuff release^[40]. Clearly, following this prescribed methodology takes some time and is prohibitive to its use within the clinical setting, however, not using these techniques can lead to inaccurate measurements, thus diluting the utility of FMD measurements. Automated analysis software may well overcome some of the difficulties regarding standardization of results^[37], however, when it is much simpler to check a cholesterol level or measure a blood pressure, it is easy to see why FMD has perhaps not yet penetrated the clinical realm. Also, FMD is strongly influenced by baseline brachial artery diameter, and changes in FMD tend to vary based on this^[41]. Finally, the absence of normal values makes it difficult to provide any clinically relevant recommendations to non-experts in the field of endothelial function assessment.

ENDOTHELIAL DYSFUNCTION IN ASYMPTOMATIC PATIENTS

In asymptomatic patients, most clinicians use the assessment of risk factors, such as the Framingham Risk score, to assess cardiovascular risk^[42]. Studies looking at the independent prognostic value of FMD in prediction of adverse events in asymptomatic patients have shown mixed

results. Suzuki *et al.*^[43], in the Northern Manhattan Study, evaluated 819 patients with cardiovascular risk factors and showed that patients with metabolic syndrome and endothelial dysfunction (measured by FMD) were at a higher risk for stroke, myocardial infarction MI or cardiovascular death than those without endothelial dysfunction. In one of the largest studies to date, Yeboah *et al.*^[44] reported that in 2792 patients with 5 years of follow up, FMD was an independent predictor of a poor outcome, however it did not appear to add much to the overall predictive model. Further large cohort studies have also shown that FMD is an independent predictor of adverse events, although there is some question as to whether the small incremental increase in prediction provided by the assessment of endothelial function mandates the routine clinical use of FMD^[31,44-46]. Indeed, other large studies have not found incremental predictive value with use of FMD. A large study of 842 asymptomatic patients in the Northern Manhattan Study found that although FMD did predict adverse outcomes it was not an independent predictor when included in a multivariable analysis including traditional cardiac risk factors^[47]. Two further studies found that while FMD was not an independent predictor of adverse events, several components of endothelial function measurement, such as hyperemic velocity and assessment of resistance artery endothelial function, were^[48,49]. In general, there is still doubt that endothelial dysfunction is a predictor of adverse cardiovascular events in asymptomatic patients.

ENDOTHELIAL DYSFUNCTION IN ESTABLISHED CAD (CHRONIC STABLE CAD)

Endothelial dysfunction in the coronary arteries is closely related to systemic endothelial dysfunction^[13]. In patients with CAD the presence of severe endothelial dysfunction has been shown to be a predictor of cardiac death, myocardial infarction or revascularization^[50]. These results have been replicated in other large studies^[51-53]. Endothelial dysfunction has also been related to adverse plaque characteristics (such as lipid-rich necrotic cores) in this group of patients^[54,55]. FMD has also been shown to be an independent predictor of in-stent restenosis in patients with single vessel coronary artery disease undergoing percutaneous coronary intervention^[56]. Elsewhere in the vascular tree, FMD has also been shown to be a predictor of post-operative MACE in patients with hypertension^[57], early peripheral arterial disease^[58] and those undergoing vascular surgery^[59].

ENDOTHELIAL DYSFUNCTION IN ACUTE CORONARY SYNDROMES

Over the past decade there has been an increasing realization that acute coronary syndromes (ACS) cannot be predicted simply by risk factors or even the presence of ob-

Table 1 Large studies evaluating the prognostic value of flow-mediated dilation

Ref.	Number of patients	Cohort	Asymptomatic Patients?	Length of follow-up (mo)	Outcome	Result	Independent value of FMD?
Rossi <i>et al</i> ^[45]	2264	Post-menopausal women	Yes	45 ± 13	CV death, MI, revascularisation, TIA, stroke	FMD was a predictor of MACE independently of traditional cardiac risk factors.	Yes
Patti <i>et al</i> ^[56]	136	Patients with single-vessel coronary artery disease undergoing PCI	No	6	In-stent restenosis	Patients with impaired FMD were more likely to suffer in-stent restenosis.	Yes
Gokce <i>et al</i> ^[59]	187	Patients undergoing vascular surgery	No	1	CV death, MI, unstable angina, ventricular fibrillation, stroke, raised troponin	FMD was an independent predictor of MACE in the immediate post-operative period.	Yes
Brevetti <i>et al</i> ^[58]	139	Patients with peripheral arterial disease	No	23 ± 10	CV death, MI, revascularisation, TIA, critical limb ischaemia	FMD was an independent predictor of events over the follow-up period.	Yes
Chan <i>et al</i> ^[53]	152	Patients with coronary artery disease	No	34 ± 10	CV death, MI, revascularisation, claudication	FMD was a strong independent predictor of risk even accounting for carotid plaque burden.	Yes
Shimbo <i>et al</i> ^[47]	842	Asymptomatic multi-ethnic cohort	Yes	36	Vascular death, MI, stroke	FMD was able to predict adverse events but not independently.	No
Suzuki <i>et al</i> ^[43]	819	Asymptomatic multi-ethnic cohort including patients with metabolic syndrome	Yes	81 ± 21	Vascular death, MI, stroke	Patients with the combination of metabolic syndrome and endothelial dysfunction had a significantly worse outcome.	No
Yeboah <i>et al</i> ^[44]	2792	Mixed cohort of patients > 65 yr	No	60	CVD death, MI, stroke, congestive heart failure, claudication, revascularisation	FMD was an independent predictor of risk but added little to traditional risk stratification.	Yes
Muiesan <i>et al</i> ^[57]	172	Hypertensive patients	No	95 ± 37	CV death, MI, revascularisation, arrhythmia, TIA, critical limb ischaemia, retinal artery occlusion	FMD below median was independently associated with adverse outcome.	Yes
Shechter <i>et al</i> ^[46]	618	Healthy subjects (mixed)	Yes	55.2 ± 21.6	CV death, MI, stroke, congestive revascularisation	FMD predicted adverse outcome independently.	Yes
Katz <i>et al</i> ^[77]	259	Heart failure patients (LVEF < 40% and NYHA class 2-3)	No	28	Death or cardiac transplantation	FMD is associated with increased adverse outcome in ischaemic and non-ischaemic heart failure.	Yes

PCI: Percutaneous coronary intervention; MACE: Adverse major cardiovascular events; MI: Myocardial infarction; TIA: Transient ischaemic attack; FMD: Flow-mediated dilation.

structive CAD^[60,61]. The development of the “vulnerable plaque” concept that leads to ACS (and sudden cardiac death) is influenced by omnipresent endothelial dysfunction *via* several methods. Endothelial dysfunction leads to reduced expression of anti-inflammatory mediators, leading to plaque destabilization^[62]. In particular, Endothelin-1, a potent vasoconstrictor, is released significantly more by the dysfunctional endothelium as well as directly at the site of unstable coronary plaque lesions^[63]. The predominant vasoconstriction of the dysfunction coronary artery may cause plaque rupture directly^[64]. Finally, the dysfunctional endothelium also has reduced anti-thrombotic tendency allowing thrombus formation^[65].

Endothelial dysfunction is also a predictor of adverse outcome in patients after ACS. Improvement of endothelial function post-ACS is associated with improved

prognosis^[66,67]. Endothelial dysfunction has also been shown to lead to adverse remodeling post-ACS^[68].

ENDOTHELIAL DYSFUNCTION IN HEART FAILURE

There is ample evidence to suggest that endothelial function is impaired in patients with both acute and chronic heart failure^[69]. NO has been shown to be involved in myocardial relaxation^[70], and reduction in NO availability (for the same reasons as seen in the vasculature) can impair left ventricular relaxation, causing diastolic dysfunction. The presence of diastolic dysfunction is associated with impaired FMD in patients with established CAD^[71]. The presence of endothelial dysfunction has also been associated with perfusion defects and reduced coronary

flow in patients with suspected coronary artery disease thus potentially leading to impaired ventricular function^[72,73]. In chronic heart failure there may be a vicious circle effect, by which the reduction of cardiac output leads to a decrease in vascular shear stress and NO production, therefore causing further worsening of endothelial function^[74]. FMD has also been shown to be a predictor of adverse outcome in heart failure patients^[75-78].

In acute heart failure, there is also a reduction in NO availability leading to vasoconstriction and increased vascular stiffness, increasing afterload. There is also increased endothelin-1 production and oxidative stress, again placing further strain on the heart and vasculature^[79,80]. Coronary artery endothelial dysfunction has been shown to predict progression of allograft vasculopathy and mortality in patients with orthotopic heart transplantation^[81,82].

Endothelial dysfunction is associated with adverse outcome in patients with LV dysfunction^[83-85]. It has also been shown to be a good predictor of response to cardiac resynchronization therapy (CRT)^[86].

CAN ENDOTHELIAL DYSFUNCTION BE REVERSED?

We have shown that there is substantial evidence to support the role of endothelial dysfunction in the development and progression of cardiovascular disease and its prognostic role. Because of this there has been a significant interest in finding methods to ameliorate endothelial dysfunction. Despite many drug classes being evaluated, only a few have shown concrete benefits on the endothelium. Large clinical studies evaluating pharmacological endothelial dysfunction reversal are summarized in Table 2.

Some of the most studied drug classes are those that act on the renin-angiotensin system, namely angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin-II receptor antagonists (ARBs). These drugs have several anti-oxidant and anti-inflammatory effects, reducing superoxide (thus reducing oxidative stress) and endothelin-1 activity^[87]. Angiotensin II stimulates angiotensin type 1 receptors (AT1) to mediate arteriolar vasoconstriction and remodelling, superoxide anion production, renal sodium reabsorption, aldosterone secretion and endothelin-1 release^[88]. Many of these actions affect the vascular endothelium adversely. On the other hand stimulation of the angiotensin type 2 (AT2) receptor by angiotensin has mainly opposing actions to those of AT1 stimulation and recently has been shown to contribute to endothelial NO release^[89]. AT2 production can be reduced by angiotensin converting enzyme inhibitors which also increase both tissue and plasma bradykinin by inhibiting kininase II^[90]. By stimulating the B2 receptors, bradykinin mediates the release of NO, prostacyclin and the endothelial hyperpolarizing factor; agents that produce vasodilation^[91-93]. The large TREND study provided evidence that quinapril was able to reverse endothelial dysfunction^[94]. The beneficial effects of ACEIs have been replicated by several other studies^[95-98]. Angiotensin-II receptor antagonists have

also shown similar results^[99,100].

Spironolactone and eplerenone, which have mineralocorticoid receptor antagonist activity have received much attention recently. They have been reported to improve NO bioactivity in patients with heart failure^[101]. The mechanism(s) by which aldosterone impairs endothelial function is unclear. Aldosterone enhances vascular responsiveness to pressor agents such as norepinephrine and angiotensin II^[102]. Also, aldosterone can cause direct vascular smooth muscle contraction *via* a non-genomic pathway that has not yet been characterised. Both drugs have however been shown to improve endothelial function in patients with heart failure and hypertension^[103-106].

Beta-blockers and diuretics have generally been shown to have no effect on endothelial function however, newer beta-blockers such as nebivolol and carvedilol have shown some beneficial effects on reversal of endothelial dysfunction^[107-109]. Nebivolol has a direct effect on NO synthase while carvedilol has some antioxidant properties. Calcium channel antagonists also improve endothelial dysfunction by several pathways, particularly in the coronary microvasculature by indirectly increasing in intracellular smooth muscle cell cGMP, which is the second messenger of NO and mediates vasodilation^[110,111]. Two additional mechanisms have been described to explain the effects of calcium channel blocker in the forearm circulation. The first explanation is that most calcium channel blockers have antioxidant activities, reducing production of superoxide anions^[88,89]. The second explanation involves a reduction in endothelin-1 release by calcium channel blockers. Endothelin-1 is a potent vasoconstrictor and it is released from the endothelium^[112]. Normally, there is a balance between vasoconstrictive and vasodilating substances in the vasculature but in hypertension, the bioavailability of endothelin might be increased in parallel with a reduction in NO bioactivity. It has shown that calcium channel blockers improved NO bioactivity by reducing endothelin release^[100,101]. In addition, Cardillo *et al.*^[113] have recently shown that in patients with essential hypertension, the increased endothelin activity is partly responsible for the increased vascular tone. Hence, in a model where vasoconstrictive activity is increased, such as hypertension, a reduction of endothelin release would improve NO bioactivity. CCBs may also improve other aspects of endothelial dysfunction, reducing tissue plasminogen activator activity, thus reducing thrombogenic risk by decreasing platelet activation^[114].

Statins also have proven beneficial effects on endothelial dysfunction in addition to their effects on lipids^[115-118]. Reduction in LDL-cholesterol is thought to be the main method by which statins improve endothelial function, however, they also enhance expression and activity of NO synthase and reduce C-reactive protein (which has deleterious effects on the endothelium)^[119,120]. On a similar, intriguing, theme of non-antihypertensive therapies improving endothelial function, recent studies have also suggested that drugs such as metformin^[121], ranolazine^[122] and allopurinol^[123] may also improve endothelial function.

Table 2 Selected studies examining pharmacological reversal of endothelial dysfunction

Ref.	Drug	Cohort	Design	Results
Mancini <i>et al</i> ^[94]	Quinapril	105 normotensive patients with coronary artery disease	Randomised double-blind, placebo controlled	Quinapril improved endothelial function compared to placebo as measured by coronary artery diameter response to acetylcholine
Higashi <i>et al</i> ^[96]	Various ACE inhibitors, beta-blockers, calcium channel blockers and diuretics	296 hypertensive patients	Multi-centre cohort study	ACE inhibitors significantly improved endothelial dependent vasodilatation compared to other drug classes as measured by forearm blood flow
Wassmann <i>et al</i> ^[97]	Candesartan, felodipine	47 patients with high cholesterol	Randomised double-blind, placebo controlled	Candesartan improved forearm blood flow compared to felodipine or placebo
Ghiadoni <i>et al</i> ^[98]	Nifedipine, amlodipine, Perindopril, telmisartan, atenolol, nebivolol	168 patients with hypertension	Randomized, single-blind, parallel-group	Only perindopril improved FMD (although perindopril, telmisartan, nifedipine and amlodipine reduced oxidative stress and increased plasma antioxidant capacity)
Tzemos <i>et al</i> ^[99]	Valsartan, amlodipine	25 hypertensive patients	Randomised double-blind, crossover	Valsartan improved forearm blood flow
Takagi <i>et al</i> ^[100]	Telmisartan	Mixed; 398 patients	Meta-analysis of 7 studies	Statistically significant increase in FMD by 48.7%
Farquaharson <i>et al</i> ^[101]	Spironolactone	10 patients with NYHA class I-II heart failure	Randomised, double-blind placebo-controlled crossover study	Spironolactone improved forearm blood flow compared to placebo
MacDonald <i>et al</i> ^[103]	Spironolactone	43 patients with NYHA class I-II heart failure	Randomised, double-blind crossover study	Spironolactone improved forearm blood flow compared to placebo
Abiose <i>et al</i> ^[104]	Spironolactone	20 patients with NYHA class III-IV congestive heart failure	Cohort study	Spironolactone improved FMD at 4 wk with a sustained improvement at 8 wk
Tzemos <i>et al</i> ^[107]	Nebivolol, atenolol	12 hypertensive patients	Randomised, double-blind crossover study	Only nebivolol was able to improve endothelial dependent vasodilation
Pasini <i>et al</i> ^[108]	Nebivolol, atenolol	40 hypertensive patients with 40 controls	Randomised double-blind parallel group	FMD improved only in the group treated with nebivolol
Matsuda <i>et al</i> ^[109]	Carvedilol	29 patients with coronary artery disease	Randomised, placebo controlled	Carvedilol significantly improved FMD after 4 mo treatment
Agewall <i>et al</i> ^[116]	Atorvastatin	20 healthy smokers, 20 healthy non-smokers	Open label placebo controlled randomised cross-over	Smokers had a lower baseline FMD. Atorvastatin improved FMD in smokers but had no effect in non-smokers
Ostad <i>et al</i> ^[117]	Atorvastatin, ezetimibe	58 patients with coronary artery disease	Double-blind, randomised, parallel group	High-dose atorvastatin improved FMD significantly more than low dose atorvastatin + ezetimibe independently of improvement in LDL cholesterol
Gounari <i>et al</i> ^[118]	Rosuvastatin, ezetimibe	Patients with heart failure	Double-blind, placebo controlled, cross-over trial	Rosuvastatin caused a significant improvement of FMD compared to ezetimibe and independent of LDL cholesterol and baseline brachial artery diameter
Pitocco <i>et al</i> ^[121]	Metformin	42 type 1 diabetics without overt cardiovascular disease	Randomised double-blind, placebo controlled	Significant improvement in FMD by 1.32% compared to placebo
Lamendola <i>et al</i> ^[122]	Ranolazine	30 type 2 (non-insulin dependent) diabetics without overt cardiovascular disease	Randomised double-blind, placebo controlled	Significant improvement in FMD compared to placebo after 2 wk of ranolazine therapy
Kao <i>et al</i> ^[123]	Allopurinol	67 patients with CKD stage 3 and LV hypertrophy	Randomized, double-blind, parallel-group	Significant improvement in FMD compared to placebo after 9 mo of allopurinol therapy

FMD: Flow-mediated dilation.

DOES REVERSAL OF ENDOTHELIAL DYSFUNCTION HAVE ANY PROGNOSTIC IMPACT?

Given that several classes of drugs do seem to lead to

an improvement in endothelial function, the next step is to consider whether these effects are translated into a prognostic benefit. There are however only a few studies which address this issue. Modena *et al*^[124] evaluated 400 post-menopausal women with hypertension and endothelial dysfunction in an attempt to assess whether

an improvement in FMD using antihypertensive drugs would predict a better prognosis. The authors found that improvement in endothelial function after 6 mo of therapy was associated with a much reduced event rate (6% *vs* 21.3% in those patients with persistently impaired endothelial dysfunction). One problem might perhaps be the fact that therapeutic options which improve endothelial function also have other beneficial effects on the cardiovascular system independent of their vasodilatory contribution. A recent study in patients with heart failure showed that patients in whom endothelial function improved following institution of optimal medical therapy had a much better prognosis than those in whom there was no improvement (hazard ratio 3.0 for those with persistently impaired endothelial function)^[78].

Furthermore, confounding effects of medications also need to be considered—for example, hormone replacement therapy with estrogens in post-menopausal women does cause vasodilatation, however this beneficial effect is negated by their pro-thrombotic tendency. Another potential role for identification of endothelial dysfunction is that of screening. Given that there is abundant evidence to suggest that endothelial dysfunction is present before the development of clinically significant cardiovascular disease it might be beneficial to identify patients at potential risk of future events and offer disease modifying therapy. Again however this question has not yet been answered.

While numerous drugs that improve endothelial dysfunction have been shown to improve mortality, very few studies have specifically looked at the beneficial prognostic effects of endothelial dysfunction. This is presumably because when designing studies investigating these drugs it is very difficult to isolate the effect of endothelial dysfunction reversal given the multi-site action of drugs such as ACE inhibitors and statins. Of course, as the beneficial effects of these drugs are now well established, trials specifically looking at the prognostic benefit of endothelial dysfunction are perhaps less of a priority.

CONCLUSION

In this review we have demonstrated the methods of endothelial function assessment, the significance of endothelial dysfunction (particularly as a precursor) to cardiovascular disease and its prognostic significance. Several aspects need further exploration. First, despite the widespread use of FMD in clinical trials, is it the best way of assessing endothelial dysfunction? Certainly, the failure of the technique to obtain widespread use in a clinical setting despite many years of use in clinical trials and a reasonable amount of prognostic evidence behind it would suggest that it may never be adopted in the cardiology community. However, the failing of FMD seems to be more due to technical issues (such as the time taken to measure it and operator variability) rather than a disbelief in its results or the importance of endothelial function. The development of PAT and interest in other aspects of endothelial function such as circulating

biomarkers relating to thrombosis and inflammation may prove to be easier methods of assessing endothelial function. If an easier method could be found then (presuming it showed similar prognostic value as FMD in large-scale studies) perhaps this would have more widespread clinical applicability. Indeed, in our unit, FMD is only used in research studies and is not used at all clinically. The standardization of the method is of key importance with regards to whether FMD can truly penetrate the clinical arena. Secondly, should endothelial dysfunction be used as an end-point to guide therapy or should it be simply thought of as another risk factor? And if so, are there any other potential therapies which might independently modulate endothelial function? Finally, does improving endothelial function lead to improved clinical outcomes in both primary and secondary prevention?

In summary, and in answer to the question posed by the title of this review, there is evidence to suggest that reversal of endothelial dysfunction might still be a target which might improve cardiovascular outcomes in the modern era, however, we do not yet have convincing evidence that it does as yet. We know that reversal is possible, but whether it is beneficial in identifying a higher risk group in primary prevention (in addition to traditional risk factors) or as a target in secondary prevention remains a question with an as yet elusive answer. It may be that FMD (and other measures of endothelial dysfunction) is more of a marker of overall cardiovascular health (predicting adverse outcome similarly to biomarkers such as B-type natriuretic peptide and troponin), rather than a therapeutic target itself. Nevertheless, there is ample evidence that therapies that improve cardiovascular outcome (by various pathways), also seem to improve endothelial function. Given the prognostic value of FMD, it would seem logical that at least some of these beneficial effects may be mediated by an improvement in endothelial function. However, as long as the most validated measurement of endothelial function (FMD) cannot reach widespread use clinically, it will remain difficult to promote the idea that reversal of endothelial dysfunction should be a primary target of treatment in its own right. Indeed, to answer the question posed in the title of this review, we believe that while reversal of endothelial dysfunction is an attractive target in modern cardiology, we still require further studies to ascertain whether directly targeting reversal of endothelial dysfunction is a worthwhile target in modern cardiology.

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Percutaneous management of vascular access in transfemoral transcatheter aortic valve implantation

Ilaria Dato, Francesco Burzotta, Carlo Trani, Filippo Crea, Gian Paolo Ussia

Ilaria Dato, Francesco Burzotta, Carlo Trani, Filippo Crea, Institute of Cardiology, Catholic University of the Sacred Heart, 00168 Rome, Italy

Gian Paolo Ussia, Department of Cardiovascular Disease, Tor Vergata University, 00133 Rome, Italy

Author contributions: Dato I, Burzotta F, Trani C, Crea F and Ussia GP contributed equally to this article.

Correspondence to: Ilaria Dato, MD, Institute of Cardiology, Catholic University of the Sacred Heart, Largo Agostino Gemelli, 8, 00168 Rome, Italy. ilariadato81@gmail.com

Telephone: +39-6-3051166 Fax: +39-6-3055535

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femoral approach and can significantly affect the overall clinical outcome. After diagnosis, the application of simple vascular interventional techniques allows efficient complication management, thus avoiding high risk vascular surgery. We discuss the available percutaneous vascular access preparation by dedicated devices, the principal diagnostic tools for prevention and detection of vascular complications and their percutaneous management in the transfemoral TAVI setting.

Dato I, Burzotta F, Trani C, Crea F, Ussia GP. Percutaneous management of vascular access in transfemoral transcatheter aortic valve implantation. *World J Cardiol* 2014; 6(8): 836-846 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i8/836.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i8.836>

Abstract

Transcatheter aortic valve implantation (TAVI) using stent-based bioprostheses has recently emerged as a promising alternative to surgical valve replacement in selected patients. The main route for TAVI is retrograde access from the femoral artery using large sheaths (16-24 F). Vascular access complications are a clinically relevant issue in TAVI procedures since they are reported to occur in up to one fourth of patients and are strongly associated with adverse outcomes. In the present paper, we review the different types of vascular access site complications associated with transfemoral TAVI. Moreover, we discuss the possible optimal management strategies with particular attention to the relevance of early diagnosis and prompt treatment using endovascular techniques.

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Key words: Transfemoral transcatheter aortic valve implantation; Vascular access complication; Percutaneous management

Core tip: Vascular complications are not rare in transcatheter aortic valve implantation (TAVI) by the trans-

INTRODUCTION

Transcatheter aortic valve implantation (TAVI) using stent-based bioprostheses has recently emerged as a promising alternative to surgical valve replacement in selected patients^[1,2]. At present, for transfemoral TAVI the most studied valves are a balloon-expandable prosthesis, the Edwards SAPIEN XT™ valve (Edwards Lifesciences, Irvine, California, United States), that has recently added to the first generation Edwards valve, the Edwards SAPIEN (and in Europe has replaced it), and a self-expandable prosthesis, the CoreValve ReValving System® (Medtronic Inc., Minneapolis, MN, United States). Percutaneous implantation is generally performed using retrograde access from the femoral artery^[3]. In spite of the increasing diffusion of TAVI across the world, with a high rate of procedural success and significant clinical and hemodynamic benefits^[4,5], procedural challenges remain relevant. Among the different procedural technical issues, femoral access management is emerging as a factor with paramount clinical relevance. Indeed, major vascular complications during TAVI may range between 5%

and 25% of patients^[6], and are associated with a striking increase in early mortality risk^[7-10].

PREDICTORS OF VASCULAR COMPLICATIONS AND SELECTION OF VASCULAR ACCESS

The rate of vascular access site complications is probably influenced by several factors, which include the size of the devices (with favorable impact expected from the reduction in sheath size required by the latest generation valves), patient anatomy and the operator's experience/technique in deploying the closure devices^[11]. Periprocedural bleeding after TAVI is frequent and principally related to renal function and sheath diameters, as reported in a recent Italian multicenter study^[12]. Life-threatening and major bleeding, along with severe kidney failure, are independent predictors of increased mortality after 30 d^[12].

While the first introduced bioprosthetic valve (Edwards SAPIEN) was characterized by a larger diameter (internal diameter 22-24 F and external diameter 8-9 mm) and required a minimal external arterial diameter of 7-8 mm, the Edwards SAPIEN XTTM valve and the Medtronic CoreValve System[®] valve which are characterized by an external diameter of about 7 mm (internal diameter 16-20 F and 18 F, respectively) necessitate a minimal external arterial diameter of about 6-7 mm (6 mm for 16 F e-Sheath and standard 18 F sheath, if ilio-femoral arteries are not severely calcified). Calcific and obstructive atherosclerosis of iliac-femoral arteries, which is common in the elderly population treated by TAVI, and small vessel diameter and tortuosity may often hinder safe positioning of large delivery catheters (16-24 F). In particular, the sheath to femoral artery ratio, independently predicts the Valve Academic Research Consortium (VARC) major vascular complications and 30-d mortality, with an identified cut-off of 1.05^[13]. Furthermore, intravascular manipulation of these large catheters increases the risk of vascular injury, even in arteries with more friendly characteristics. Therefore, an accurate, pre-interventional screening of vascular anatomy using angiography or multidetector computed tomography (MDCT) of iliac-femoral arteries is mandatory for TAVI, to assess the presence and severity of atherosclerotic disease and determine the feasibility of an arterial approach^[14]. Ideally, iliac-femoral arteries should be free of heavily calcified plaques and significant tortuosity, and with a diameter large enough to accommodate a large femoral sheath^[13,15]. In comparison with standard angiography, the multiplanar capabilities of MDCT allow a detailed and complete three-dimensional assessment of the iliac-femoral system^[16]. In addition to the accurate measurement of minimal lumen diameters, MDCT can assess vessel tortuosity, burden and pattern of calcification, extent of atherosclerosis, and identify other high-risk features including dissections and complex atheroma. During the procedure, fluoroscopic guidance while advancing the large diameter sheaths and delivery catheters

is mandatory in order to check their navigation through complex vessel features. Ultrasound (US) guidance during positioning of these devices can help in identifying the optimal common femoral artery (CFA) puncture site and has been suggested to reduce access site complications^[17]. In a multicenter randomized controlled trial, routine real-time US guidance compared with standard fluoroscopic guidance improved CFA cannulation only in patients with high CFA bifurcations, but improved first-pass success rate and reduced the number of attempts, time to access, risk of venipuncture, and vascular complications in all cases^[18].

HEMOSTASIS TECHNIQUES USED IN TAVI

After an initial phase of surgical access site preparation and closure of vascular access, which is still to be considered in particular cases of alternative access (*e.g.*, transsubclavian access), operators have become confident with percutaneous puncture and access site closure through commercially available suture-mediated closure devices, such as the Prostar XL10F and Perclose ProGlide (Abbott Vascular Devices, Redwood City, CA, United States) devices^[19,20]. Classical surgical preparation of vascular access can be quite difficult and time-consuming, especially in patients with heavily calcified vessels and/or previous groin interventions. It is characterized by a circumferential vessel dissection, arteriotomy, clamping, and wall closure. In all these phases vascular access complications such as plaque disruption, local dissection, aneurysm formation, stenosis/occlusion, and even acute thrombosis, with consequent acute limb ischemia, can occur^[21,22]. Moreover, the lesser invasive percutaneous method in an experienced center is associated with similar rates of major and minor vascular complications^[23] and with lower access site infection and bleeding, and shorter hospital stay compared to the surgical approach^[24].

While the Edwards SAPIEN valve is implanted through a 22 or 24 F arterial sheath (about 8 and 9 mm external diameter), the CoreValve and the Edwards SAPIEN XT valve are delivered through a 16-20 F sheath (about 7 mm external diameter). These bulky sheaths are above the "on label" use of both suture-based hemostatic devices like the Prostar XL and Perclose ProGlide. So the "preclosure" technique has been developed to allow achievement of a full percutaneous hemostasis using such devices. The "preclosure" technique is based on the application of these devices to deploy sutures before the introduction of the large arterial sheath needed for valve implantation, then the sutures are tied at the end of the procedure by pushing down knot(s) in order to achieve hemostasis percutaneously. The sequence of steps necessary for successful "preclosure" technique is depicted in Figure 1. Recently, Kahlert *et al.*^[25] reported that "preclosure" with a single ProGlideTM device, followed by manual compression, could provide a more efficient and safe hemostasis compared to multiple ProGlideTM and Prostar

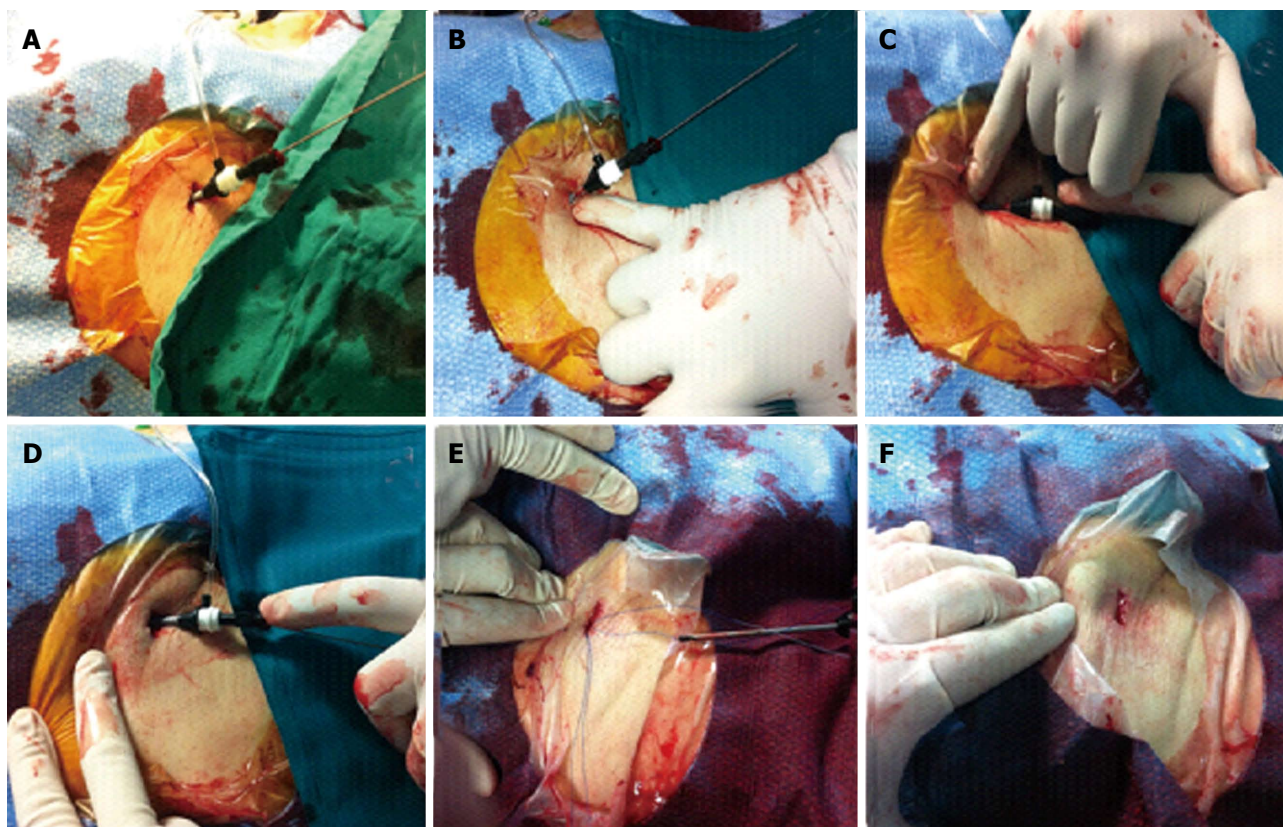


Figure 1 Pre-closure technique for hemostasis in transcatheter aortic valve implantation procedures. After angiography-guided puncture of the anterior wall of the common femoral artery (CFA) and the insertion of a 6 F sheath, the preparation of vascular access for large sheath insertion (≥ 18 F) consists of the enlargement of the access site by the insertion of a 9 F sheath (A) and dilation of the subcutaneous tissue anteriorly (B) and posteriorly to the sheath (C), using one finger. Such a maneuver should achieve a less traumatic flaring of cutaneous and subcutaneous tissues at the vascular access site and create appropriate space for both large sheath introduction at the beginning of the procedure and optimal fastening of knots over the arterial wall at procedure end (D). After 9 F sheath removal, the suture-mediated vascular closure device is inserted in the correct position, the needles are unlocked and pulled through the arterial wall (E). At the end of transcatheter aortic valve implantation, the sheath and the guide wire are removed, the sutures are fastened individually with a sliding knot and a knot pusher is used to ensure approximation of the knot to the surface of the vessel wall. Vascular suture ends are cut well beneath the surface of the skin and an optimal closure of vascular access is obtained by a single cutaneous suture without residual bleeding (F).

XL techniques.

The Prostar XL device was originally designed for a suture-based 10 F arteriotomy closure. However, it is commonly used for closing arterial access sites up to 18 F using the preclosure technique^[26]. The device is a suture-mediated vascular closure system and is composed of a 10 F, 0.038-inch guidewire-compatible, hydrophilic sheath with a J-tip and a monorail design, based on two sutures (USP 3-0 braided polyester) and two pairs of nitinol needles, a needle guide, and a rotating barrel precisely controlling the needles during device deployment. After angiography-guided puncture of the anterior wall of the common femoral artery at an angle of approximately 45°, the Prostar XL is advanced over a 0.035-inch guidewire. When the device is in the correct position, indicated by pulsatile blood return from the dedicated marker lumen, the needles are unlocked and pulled through the arterial wall. After deployment of the device, the sutures are secured with mosquito clamps. At the end of the TAVI procedure, the sheath and the guide wire are removed while proximal pressure is maintained, and sutures are fastened individually with a (manually performed) sliding knot. A knot pusher is used to ensure approximation of the knot to the surface of the vessel wall. Manual pres-

sure is then released and suture ends are cut well beneath the surface of the skin. A single Prostar XL is generally used to close arteriotomies for 18 to 19 F sheaths and two devices for 22- and 24 F sheaths at a 45° angle. It has been demonstrated to be a safe and effective method of achieving hemostasis, and to reduce times to ambulation and discharge after interventional procedures in a multicenter, non-randomized registry^[26].

The Perclose ProGlide is a 6 F suture-based hemostatic device consisting of a monofilament suture and a pre-formed knot. To obtain hemostasis after removal of large sheaths, two Perclose devices are used according to the “double preclosure technique”. This consists of the sequential insertion of the two Perclose devices rotated in opposite sides at 30°-45°, to create an interrupted X-figure and then closure of the arteriotomy is achieved at the end of the procedure by tying down the two knots using the two node pushers sequentially^[27]. According to recent data, this technique has been suggested to be associated with a low incidence of early and late closure site complications^[28-30]. Furthermore, the use of three Perclose devices has recently been reported^[19].

Finally, a potentially useful adjunctive technique (which may eventually be used in conjunction with the above-

Table 1 Valve academic research consortium-2 classification of vascular access site and access-related complications

Major vascular complications
Any aortic dissection, aortic rupture, annulus rupture, left ventricle perforation, or new apical aneurysm/pseudoaneurysm OR
Access site or access-related vascular injury (dissection, stenosis, perforation, rupture, arterio-venous fistula, pseudoaneurysm, hematoma, irreversible nerve injury, compartment syndrome, percutaneous closure device failure) leading to death, life-threatening or major bleeding ¹ , visceral ischemia or neurological impairment OR
Distal embolization (non-cerebral) from a vascular source requiring surgery or resulting in amputation or irreversible end-organ damage OR
The use of unplanned endovascular or surgical intervention associated with death, major bleeding, visceral ischemia or neurological impairment OR
Any new ipsilateral lower extremity ischemia documented by patient symptoms, physical exam, and/or decreased or absent blood flow on lower extremity angiogram OR
Surgery for access site-related nerve injury OR
Permanent access site-related nerve injury
Minor vascular complications
Access site or access-related vascular injury (dissection, stenosis, perforation, rupture, arterio-venous fistula, pseudoaneurysms, hematomas, percutaneous closure device failure) not leading to death, life-threatening or major bleeding ¹ , visceral ischemia or neurological impairment OR
Distal embolization treated with embolectomy and/or thrombectomy and not resulting in amputation or irreversible end-organ damage OR
Any unplanned endovascular stenting or unplanned surgical intervention not meeting the criteria for a major vascular complication OR
Vascular repair or the need for vascular repair (<i>via</i> surgery, ultrasound-guided compression, transcatheter embolization, or stent-graft)
Percutaneous closure device failure
Failure of a closure device to achieve hemostasis at the arteriotomy site leading to alternative treatment (other than manual compression or adjunctive endovascular ballooning)

¹Refers to valve academic research consortium bleeding definitions^[37].

mentioned closure device-based techniques) to improve efficacy of hemostasis, is the crossover balloon occlusion technique (CBOT). This consists of the reduction of local blood pressure at the entry level of the large sheath through flow blockage obtained by inflation of a peripheral angioplasty balloon in the iliac artery using the crossover technique. The CBOT has been reported to allow safe and successful percutaneous closure in patients undergoing TAVI *via* a retrograde femoral artery approach using the 22 or 24 F sheath systems^[31].

NOVEL VASCULAR SHEATHS FOR TRANSFEMORAL TAVI

More recently, a novel type of sheath has been developed to reduce the rate of vascular complications related to TAVI. The SoloPath™ (Onset Medical, Terumo Medical Corporation, Irvine, CA, United States) is a balloon expandable transfemoral introducer; it has an inner diameter of 14-21 F (outer diameter 17-24 F) and is compatible with the 18 F Medtronic/CoreValve and the 23- and 26-mm Edward SAPIEN XT delivery system. Its peculiarity is represented by a 13.5 F distal part to facilitate vessel entry, that can be expanded by the integrated balloon inflation reaching its nominal diameter, after sheath insertion, and can be deflated at the end of the procedure, enabling low-resistance removal^[32,33]. The SoloPath sheath is a feasible alternative to conventional sheaths for transfemoral TAVR patients with advanced atherosclerotic disease or an arterial diameter ≤ 7 mm^[34]. The available expandable sheath for Edwards Sapien XT valve is the e-Sheath™ (Edwards Lifesciences, Irvine, California, United States), a 16-18 F sheath, with a “dynamic expansion mechanism” to facilitate the valve passage, which returns to a reduced profile once the valve has passed, limiting vascular trauma. Nevertheless, this device is con-

traindicated for tortuous or calcified vessels, which would prevent safe entry of the sheath, and currently does not show an advantage over the 18/19 F fixed size sheath in reducing vascular and bleeding complications^[35].

VASCULAR ACCESS SITE COMPLICATIONS AFTER TAVI AND THEIR MANAGEMENT

A series of vascular complications are commonly reported to be associated with TAVI, including arterial perforation, dissection, pseudoaneurysm, stenosis/occlusion and arterio-venous fistula^[7-10]. The VARC, a collaboration between academic research organizations in the United States and Europe, has elaborated a consensus document on TAVI related endpoint definitions^[36] and a more recent updated document^[37], in which a classification of major and minor vascular access complications has been proposed (Table 1). This position paper has also provided a clear definition for the “access-related” complications, which were defined as any adverse clinical event possibly associated with any of the access sites used during the procedure^[38].

Vascular access site complication rates reported in the literature are extremely variable probably because of different valve delivery systems^[39], closure techniques and learning curves. To provide an overview of vascular complication frequency and type, a summary of the main published studies on TAVI-related vascular access site complications is provided in Table 2.

Optimization of hemostasis techniques and management strategies are probably pivotal. The optimal management of vascular access site complications includes a prompt diagnosis and appropriate timely treatment. At the end of the procedure, digital subtraction angiography of the iliac-femoral arteries obtained using a non-selec-

Table 2 Incidence of major vascular access site complications and specific vascular access site types across transfemoral transcatheter aortic valve implantation studies

Ref.	Bioprostheses	Population	Major vascular complications	Stenosis/occlusion	Perforation/rupture	Dissection	Pseudoaneurysm	
Webb <i>et al</i> ^[40] , <i>Circulation</i> 2009	ESV	113	9/113 (8%)	NA	NA	NA	NA	
Ducrocq <i>et al</i> ^[8] , <i>Eurointervention</i> 2010	ESV	54	9/54 (16.7%)	0/9	5/9 (55.5%)	4/9 (45.5%)	0/9	
Tchetché <i>et al</i> ^[9] , <i>Eurointervention</i> 2010	ESV + MCV	45	4/45 (8.9%)	NA	NA	NA	NA	
		24 ESV	2/24 (8.3%)					
		21 MCV	2/21 (9.5%)					
Piazza <i>et al</i> ^[41] , <i>Eurointervention</i> 2008	MCV	646	12/646 (1.9%)	NA	NA	NA	NA	
Himbert <i>et al</i> ^[42] , <i>JACC</i> 2009	ESV	51	6/51 (12%)	0/6	2/6 (33%)	4/6 (66%)	0/6	
Webb <i>et al</i> ^[1] , <i>Circulation</i> 2007	ESV	50	4/50 (8%)	0/4	4/4 (100%)	0/4	0/4	
SOURCE registry ^[43] , <i>Circulation</i> 2009	ESV	463	57/463 (12.3%)	NA	NA	NA	NA	
Lefèvre <i>et al</i> ^[44] , <i>Eur Heart J</i> 2011	ESV	61	17/61 (28%)	0/61	3/61 (5%)	6/61 (10%)	1/61 (2%)	
Canadian experience ^[45] , <i>JACC</i> 2010	ESV	168	22/168 (13.1%)	NA	NA	NA	NA	
Bleiziffer <i>et al</i> ^[46] , <i>J Thorac Cardiovasc Surg</i> 2009	MCV	153	24/153 (16%)	NA	NA	NA	NA	
The Milan experience	ESV + MCV	107	22 /107 (20.6%)	13/61	1/22 (4.5%)	7/22 (32%)	6/22 (27%)	4/22 (18%)
<i>JACC Cardiovasc Interv</i> 2010 ^[47]		61 ESV	(21.3%)	ESV	5/13 (38%)	ESV	4/13 (31%)	
		46 MCV	9/46 (19.5%)		2/9 (22%)	MCV ¹	2/9 (22%)	MCV ¹
The Rotterdam experience ^[7] , <i>Eurointervention</i> 2010	MCV	99	13/99 (13%)	NA	NA	NA	NA	NA
The France Registry ^[48] , <i>Eur Heart J</i> 2011	ESV + MCV	160	11/160 (7%)	0/11	2/11 (18%)	ESV	7/11 (64%)	0/11
		94 ESV	6/94 (6.4%)			ESV	4/6 (67%)	
		66 MCV	(7.6%)			MCV ¹	3/5 (60%)	MCV ¹
Petronio <i>et al</i> ^[49] , <i>Circ Cardiovasc Interv</i> 2010	MCV	460	9/460 (2%)	NA	NA	NA	NA	NA
Spanish experience ^[50] , <i>Rev Espan Cardiol</i> 2010	MCV	108	6/108 (5.6%)	1/6 (16.6%)	1/6 (16.6%)	0/6	1/6	(6.60%)
United Kingdom Registry ^[51] , <i>JACC</i> 2011	ESV + MCV	599	50/599 (8.4%)	NA	NA	NA	NA	NA
		193 ESV						
		406 MCV						
Toggweiler <i>et al</i> ^[15] , <i>JACC</i> 2012	ESV + MCV	137	24/137 (18%) ²	16/24	2/24 (8.3%)	2/24 (8.3%)	2/24 (8.3%)	2/24 (8.3%)
		126 ESV		(66.6%)				
		11 MCV						
Partner trial ^[52] , <i>JACC</i> 2012	ESV	419	64/419 (15.3%)	NA	20/64 (31.3%)	40/64 (62.8%)	2/64 (3.4%)	
The France II Registry ^[53] , <i>NEJM</i> 2013	ESV + MCV	3195	150/3195 (4.7%)	NA	NA	NA	NA	
		2107 ESV	57/2107 (2.7%)					
		1043 MCV	47/1043 (4.5%)					
European Sentinel Registry of TAVI ^[54] , <i>Eurointervention</i> 2013	ESV + MCV	4571	40/4571 (3.1%)	NA	NA	NA	NA	
		2604 ESV ³	20/2604 (3.3%)					
		1943 MCV	20/1943 (2.8%)					
Sawa <i>et al</i> ^[55] , <i>Circulation Journal</i> 2014	MCV	44	5/44 (11.54%)	NA	NA	NA	NA	
Spanish National Registry of TAVI ^[56] , <i>Rev Esp Cardiol</i> 2013	ESV + MCV	1159	42/1159 (3.6%)	NA	NA	NA	NA	
		504 ESV	25/504 (5%)					
		610 MCV	17/610 (2.8%)					
Total		12862	640/12862 (5%)	18/143 (12.6%)	44/207 (21.2%)	69/207 (33.3%)	10/207 (4.8%)	

¹P value not significant between ESV and MCV subgroups; ²Major plus minor complications; ³Including transapical (29% of total ESV). ESV: Edwards SA-PIEN valve; MCV: Medtronic Core Valve.

tive (*via* a pigtail catheter introduced in the aorta through the contralateral femoral artery) or a selective (*via* a diagnostic right Judkins or internal mammary artery catheter placed from the contralateral femoral artery according to the “crossover” technique) contrast injection is advisable to assess the vascular integrity and promptly manage possible complications. Percutaneous management of vascular complications after TAVI as a bailout procedure

is feasible and safe, with a high rate of technical success, and long-term clinical outcomes are comparable to patients without vascular complications^[57].

A wide range of vascular damage (from minor vessel complications such as localized femoral artery dissection to major complications such as vessel occlusion or perforation) has been described. Localized vascular damage without any impairment of lower limb perfusion should

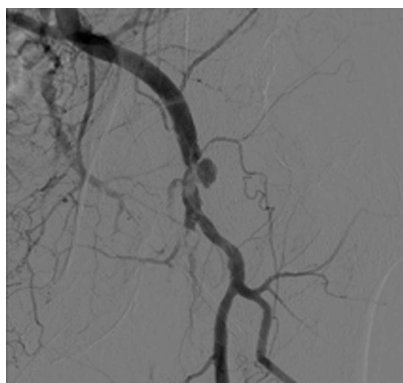


Figure 2 Post-transcatheter aortic valve implantation pseudoaneurysm. After the transcatheter aortic valve implantation procedure, digital subtraction angiography of the left iliac-femoral artery by contralateral medium contrast injection showing a pseudoaneurysm of the left common femoral artery.

be treated conservatively, with careful clinical and ultrasonographic monitoring during the following hours. The main vascular access site complications reported in TAVI studies are: pseudoaneurysm, arterial perforation, arterial dissection, occlusion and avulsion. The specific management strategies are herein discussed for each of these complications.

Pseudoaneurysm

Pseudoaneurysm consists of a pulsatile hematoma which communicates with an artery through a disruption in the arterial wall. At the end of the procedure, standard or digital subtraction angiography of the iliac-femoral arteries can reveal an arterial leak as a precursor of the pseudoaneurysm or a true pseudoaneurysm (as shown in Figure 2), depending on the time of formation. If angiographic diagnosis has not been made after the end of the procedure, close clinical surveillance can detect the increase in a new thrill or bruit, pulsatile hematoma, or marked pain or tenderness, and pseudoaneurysm can be confirmed by ultrasound. Possible complications of pseudoaneurysm are rupture, distal embolization, infection, neuropathy and local skin ischemia. However, it generally does not impair lower limb perfusion and can be treated by ultrasound-guided compression, which is a safe and cost-effective method of achieving pseudoaneurysm thrombosis^[58]. However, it carries considerable drawbacks including long procedure times, patient discomfort and high recurrence rates, especially in cases requiring anticoagulant therapy. If probe compression fails, treatment options include ultrasound-guided thrombin injection, which is associated with a high success rate and is more comfortable for patients^[59], coil embolization, stent graft and surgical repair.

Another vascular complication of TAVI is iliac-femoral stenosis, which is sometimes associated with closure device release. Mild stenosis detected by angiography in the absence of lower limb ischemia may be managed conservatively, while a significant stenosis may be treated by percutaneous transluminal angioplasty (PTA) (Figure 3), with the aim of preventing further flow deterioration

in the limb by superimposition of thrombosis or development of severe post-procedural claudication. When hemostatic device-induced tight stenosis is detected immediately after large sheath removal and urgent PTA is needed at procedure end, the selection of undersized peripheral balloons is advisable in order to avoid arterial wall laceration by suture knots.

Perforation

Perforation leading to retroperitoneal hematoma is a dramatic complication of TAVI. It can be identified by angiography performed before removal of the large sheath or can appear only after sheath removal (since the sheath is usually occlusive at the level of the external iliac and femoral arteries), as well as after tying the closure device knots. After arterial perforation visualization by angiography, timely bleeding control may be obtained by the positioning of an occlusive balloon proximal to the vascular lesion site or insertion of a large sheath across the lacerated segment. To facilitate bleeding control, operators can use protamine to neutralize heparin action. If arterial laceration persists after balloon or sheath removal, percutaneous implantation of a covered stent can be performed in order to avoid the risks related with urgent vascular surgery (Figure 4). Moreover, post-procedure digital subtraction angiography of the iliac-femoral arteries can also allow detection of rarer complications with insidious diagnosis such as lateral circumflex femoral artery perforation. While femoral artery perforation is most often related to closure device failure and can cause a visible leg hematoma, iliac artery perforation may cause a retroperitoneal hematoma in the hours after the procedure, which may be suggested by low back pain and can be confirmed by CT, and can be managed by prolonged balloon inflation or coil embolization.

Dissection

Dissection of the iliac-femoral arteries can occur as a consequence of excessively traumatic sheath insertion through fragile/diseased arterial vessels. Limited, non-occlusive and retrograde arterial dissections may generally be managed conservatively, since the antegrade flow generally maintains the artery patency, pushing the dissection flap to the vessel wall. More extensive arterial dissection can be associated with vessel occlusion (due to superimposed acute thrombosis or obstructive flaps), and may cause acute limb ischemia, so prompt management is needed to restore antegrade flow. Percutaneous angioplasty and self- or balloon-expandable stent implantation can allow successful management by the crossover technique through the contralateral femoral artery (Figures 5 and 6). A valuable tip to reduce the incidence of vascular wall lacerations is to pay particular attention to vascular calcification movement during a large sheath insertion. If the operator notes a certain resistance during this maneuver, it is advisable to insert the sheath slowly stopping every two centimeters, and to use a substance to reduce friction such as sterile Vaseline. At the end of TAVI, extraction of the introducer after dilator insertion is prefer-

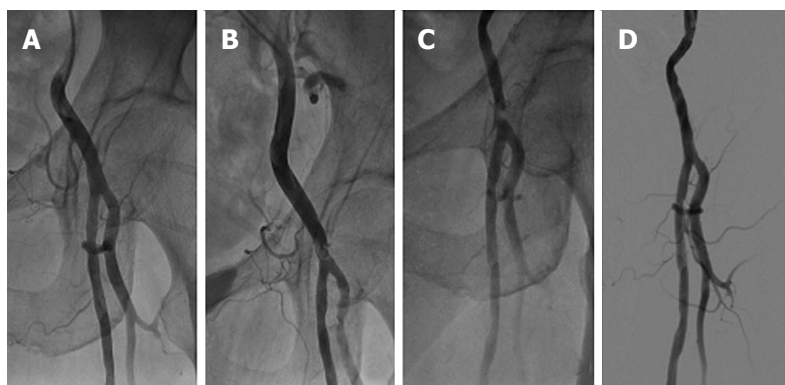


Figure 3 Post-transcatheter aortic valve implantation common femoral artery stenosis. Standard angiography obtained before 18 F sheath insertion for transcatheter aortic valve implantation showed the absence of significant stenosis, tortuosity and calcification of left iliac-femoral artery (A); after vascular access closure by Prostar XL, angiography documented the presence of an intimal flap in the right common femoral artery (CFA) wall, not determining a significant flow limitation (B); 4-mo follow-up angiography showed progression of arterial damage and the development of significant stenosis of CFA, determining claudication (Fontaine-Leriche class IIb) (C); angioplasty of left CFA was performed by right transradial access, using a 125 cm 6 F Multipurpose guiding catheter and a 300 cm BMW Universal wire; a 4.0 mm x 15 mm non-compliant coronary balloon (NC Sprinter, Medtronic, North Carolina, United States) and a 6.0 mm x 20 mm peripheral balloon (Avion Plus, Invatec, Roncadelle, Italy) were inflated to 24 atm, obtaining an optimal final result (D).

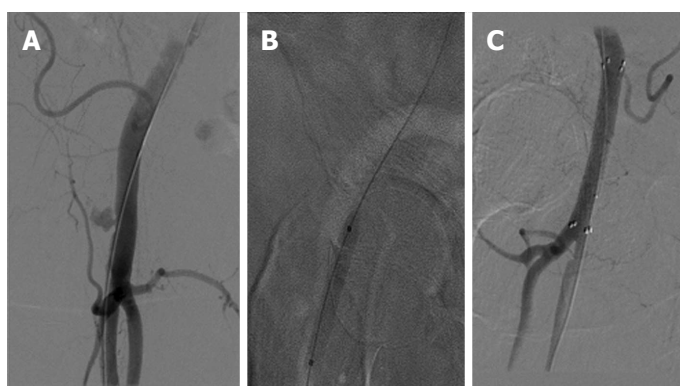


Figure 4 Post-transcatheter aortic valve implantation arterial perforation. At the end of the transcatheter aortic valve implantation procedure, digital subtraction angiography of the right iliac-femoral artery showed a perforation of the right common femoral artery (CFA) (A); angioplasty of the right CFA was performed by the crossover approach via the contralateral iliac-femoral artery; a 7.0 mm x 40 mm peripheral balloon (Admiral Xtreme, Invatec, Roncadelle, Italy) was inflated to 10 atm at the perforation site (B); because of the persistence of hematic extravasation, a 8.0 mm x 60 mm covered stent (Fluency Stent-Graft, BARD Peripheral Vascular, AZ, United States) was implanted, followed by dilation of 7.0 mm x 40 mm and 8.0 mm x 20 mm balloons (Admiral Xtreme, Invatec, Roncadelle, Italy) to 12 atm. At final angiography, optimal sealing of the arterial breach without residual hematic extravasation was documented (C).



Figure 5 Post-transcatheter aortic valve implantation arterial dissection. Post-transcatheter aortic valve implantation procedure, angiography of the right iliac-femoral axis via the contralateral groin showing a dissection of the right common femoral artery extending proximally to the external iliac artery and determining distally an occlusion of the superficial femoral artery (A); digital subtraction angiography after reaching true lumen by a .035" wire by the retrograde approach and peripheral balloon dilation (6.0 mm x 120 mm Admiral Xtreme, Invatec, Roncadelle, Italy) to 6 atm (B); final angiography after stenting (6.0 mm x 80 mm and 9.0 mm x 60 mm Lifestent Vascular Stent, BARD Peripheral Vascular, AZ, United States) and post-dilation (5.0 mm x 80 mm and 6.0 mm x 120 mm Admiral Xtreme, Invatec, Roncadelle, Italy) showing an optimal antegrade flow in the right iliac-femoral artery (C).

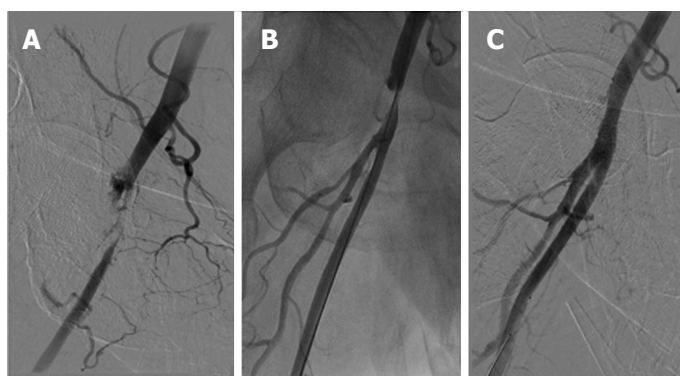


Figure 6 Post-transcatheter aortic valve implantation arterial thrombosis. Post-transcatheter aortic valve implantation procedure, digital subtraction angiography showing acute thrombotic occlusion of the right common femoral artery (A); emergency percutaneous transluminal angioplasty was performed by the crossover approach via the contralateral femoral artery, and consisted of initial thromboaspiration using a 6 F Multipurpose guiding catheter (Vista Brite Tip, Cordis Inc., Miami Lakes, FL, United States), obtaining restoration of antegrade blood flow (B); after prolonged dilations by 5.0 mm x 40 mm and 6.0 mm x 40 mm balloons (Pacific Xtreme and Admiral Xtreme, Invatec, Roncadelle, Italy), a 7.0 mm x 20 mm stent (Cristallo Ideale, Invatec, Roncadelle, Italy) was implanted, dilated by a 7.0 mm x 30 mm balloon (Avion Plus, Invatec, Roncadelle, Italy) to 10 atm. Final angiography showed the absence of residual stenosis (C).

Table 3 Materials for bailout endovascular interventions to manage vascular access complications (through contralateral femoral access using the “crossover” technique)

Complication	Type of bailout endovascular intervention	Devices needed
Any type	Immediate angiography and prompt access to the affected iliac-femoral axis ¹	6-9 F long (45 cm) sheaths
Iliac-femoral arteries rupture/perforation	Immediate hemostasis to avoid shock	Large peripheral balloons in iliac arteries (diameter: 7-10 mm) or elastomeric balloon in the distal aorta
	Vascular sealing in case of persistent blood extravasation after prolonged balloon inflation	Covered stent (diameter: 7-10 mm)
Failure of hemostasis at the entry site	Prolonged balloon inflation proximal to the entry site during external manual compression	Mid-sized peripheral balloons (diameter: 6-8 mm)
Iliac-femoral arteries flow-limiting dissection	Immediate restoration of antegrade flow to avoid acute limb ischemia	Large peripheral balloons (diameter: 7-10 mm)
	Vascular sealing in case of significant stenosis/dissection after balloon inflation	Peripheral self-expandable nitinol stents (diameter: 7-10 mm)
Iliac-femoral arteries acute thrombotic occlusion	Immediate restoration of antegrade flow to avoid acute limb ischemia	Thrombus aspiration with thrombus-extraction devices (angiojet, thrombus-aspirating catheters) or with coronary guiding catheters (multipurpose curve)
		Peripheral balloons (diameter: 5-10 mm)
		Consider distal filter protection to avoid embolization and avoid aggressive dilations since dethrombosis is usually facilitated by antegrade flow restoration

¹Provisional delivery of a sentinel wire (*i.e.*, a 0.014”-0.018” wire placed in cross-over in distal femoral artery and jailed under the 18 F sheath) allows continuous control of the entry site and quick access to contralateral iliac-femoral axis if needed.

able to avoid traumatic action of the introducer’s tip on arterial walls, especially in sharp arterial turns.

A rare complication of large artery sheath use is arterial avulsion followed by massive hemorrhage. This event is related to the tendency of the large femoral sheath to adhere to endothelium. If there is a suspicion of this dreadful complication due to resistance in sheath withdrawal, the placement of an occlusive balloon in the abdominal aorta under the renal arteries and preparation for possible surgical repair is the only option to save the patient’s life^[60].

A particular category of vascular access complications is represented by closure device failure, which is considered separately in the new VARC-2 classification^[37]. Vascular closure device failure is not uncommon and can cause arterial dissection, perforation and occlusion. For example in a study by Van Mieghem *et al*^[7], in the setting of transfemoral TAVI using the Medtronic CoreValve prosthesis, Prostar XLTM failure was responsible for about 54% of the observed major vascular events. Patient characteristics such as excessive femoral artery calcification, female gender and obesity^[61], and the operator’s learning curve^[62] in deploying the closure devices can contribute to these events. As for the other vascular complications, closure-related complications can be managed conservatively by manual compression if there is no impairment of blood flow and leg perfusion, vice versa if there is continuous access site bleeding or significant artery stenosis or occlusion, they can be treated interventionally by PTA.

As discussed above, the prompt adoption of simple endovascular techniques may help to manage the majority of vascular complications, thus avoiding the risks of urgent vascular surgery. In Table 3 an “operative” list of the endovascular materials which may be used for bailout

endovascular interventions (through contralateral femoral access using “crossover” technique) is provided.

CONCLUSION

Vascular complications are not rare in TAVI by the trans-femoral approach and can significantly affect the overall clinical outcome^[8-10]. At the end of the TAVI procedure, a control angiography obtained from the contralateral femoral access site allows early identification of vascular access site complications. After diagnosis, the application of simple vascular interventional techniques allows efficient complication management, thus avoiding high risk vascular surgery.

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Pseudoexfoliation syndrome and cardiovascular diseases

Georgios K Andrikopoulos, Dimitrios K Alexopoulos, Sotirios P Gartaganis

Sotirios P Gartaganis, Georgios K Andrikopoulos, Department of Ophthalmology, University of Patras Medical School, 26504 Patras, Greece

Dimitrios K Alexopoulos, Department of Internal Medicine, Division of Cardiology, University of Patras Medical School, 26504 Patras, Greece

Author contributions: Andrikopoulos GK, Alexopoulos DK and Gartaganis SP contributed to this paper.

Correspondence to: Sotirios P Gartaganis, MD, Emeritus Professor, Department of Ophthalmology, University of Patras, Medical School, 26504 Rion, Patras, Greece. s.gartag@med.upatras.gr

Telephone: +30-61-0271647 Fax: +30-61-0271647

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Abstract

Pseudoexfoliation (PEX) syndrome is a well-recognized late-onset disease caused by a generalized fibrilloglycopathopathy. It is linked to a broad spectrum of ocular complications including glaucoma and perioperative problems during cataract surgery. Apart from the long-known intraocular manifestations, PEX deposits have been found in a variety of extraocular locations and they appear to represent a systemic process associated with increased cardiovascular and cerebrovascular morbidity. However, as published results are inconsistent, the clinical significance of the extraocular PEX deposits remains controversial. Identification of PEX deposits in the heart and the vessel wall, epidemiologic studies, as well as, similarities in pathogenetic mechanisms have led to the hypothesis of a possible relation between fibrillar material and cardiovascular disease. Recent studies suggest that PEX syndrome is frequently linked to impaired heart and blood vessels function. Systemic and ocular blood flow changes, altered parasympathetic vascular control and baroreflex sensitivity, increased vascular resistance and decreased blood flow velocity, arterial endothelial dysfunction, high levels of plasma homocysteine and arterial hypertension have all been demonstrated in PEX subjects. Common features in the pathogenesis

of both atherosclerosis and PEX, like oxidative stress and inflammation and a possible higher frequency of abdominal aorta aneurysm in PEX patients, could imply that these grey-white deposits and cardiovascular disorders are related or reflect different manifestations of the same process.

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Key words: Pseudoexfoliation; Cardiovascular disease; Cerebrovascular disease; Coronary artery disease; Homocysteine

Core tip: Although much remains to be clarified concerning causes, pathogenesis and systemic role of pseudoexfoliation aggregations, there is accumulating epidemiologic, clinical and laboratory evidence that this well-described clinical entity may occur as part of a systemic disorder with cardiovascular implications. The present review aims to summarize current knowledge on cardiovascular complications which have been associated with these suspicious whitish-gray deposits.

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INTRODUCTION

Pseudoexfoliation (PEX) syndrome is an age-related disorder characterized by accumulation and deposition of microfibrillar material on multiple ocular and extraocular structures (Figure 1). The definite clinical diagnosis of the syndrome is based on slit lamp observation of the whitish flake-like deposits on anterior segment structures, particularly on the anterior lens surface and the pupillary border of the iris.

PEX syndrome is the most common identifiable

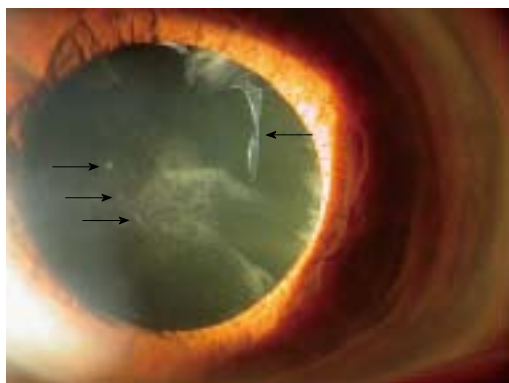


Figure 1 Pseudoexfoliation material on the anterior lens surface.

cause of open angle glaucoma, the so-called PEX glaucoma. It is also associated with cataract progression and intraoperative complications like zonular or posterior capsule rupture, poorly dilating pupil, vitreous loss, fibrinoid reaction, as well as, luxation of intraocular lens implants and corneal endothelial decompensation. In addition to the structures of the anterior segment of the eye, similar deposits have been identified in various visceral organs such as lung, heart, brain, vessels, kidney, gallbladder and meninges with unknown clinical significance.

PEX syndrome's prevalence demonstrates considerable geographic, ethnic and racial variation. Low PEX syndrome rates (< 6% in patients older than 70 years) have been reported in Greenland Eskimos, India, the eastern part of the United States, Germany, Britain, Australia, Japan, Austria, Denmark and Switzerland. In contrast, high PEX syndrome frequencies (> 15%) have been reported in Iceland, Finland, Russia, Tunisia, Saudi Arabia, Sweden, Norway, Turkey and Greece^[1-3].

Although specific synthesis and pathogenesis of PEX material are still unknown, the concept of an elastotic process has recently been established. Molecular and biochemical data support the pathogenetic concept of PEX as a type of stress-induced elastic microfibrilopathy. PEX etiopathogenesis involves both genetic and non-genetic factors. Single-nucleotide polymorphisms (SNPs) in the coding region of the lysyl-oxidase-like 1 (*LOXL1*) gene, which is responsible for cross-linking of elastin, have been identified as strong genetic risk factors for PEX syndrome and PEX glaucoma^[4]. Moreover, non-genetic factors including ultraviolet light exposure, dietary factors, infectious agents and trauma, as well as, oxidative stress, hypoxia and inflammation have been suggested to act as co-modulating external factors^[5]. Pro-fibrotic cytokines (Interleukin-6), growth factors (GFs) and particularly transforming growth factor- β 1 (TGF- β 1), impaired cellular protection system with increased cellular and oxidative stress, a change in the local balance between Matrix Metalloproteinases and Tissue Inhibitor of Metalloproteinases appear to be involved in the disorder of the fibrotic matrix with accumulation of extracellular material. Ischemia/hypoxia, cross-linking mechanisms and aggregation of misfolded stressed proteins, as well as, low-grade chronic inflammatory processes have also been

implicated^[6-9]. PEX material seems to represent a highly cross-linked glycoprotein-proteoglycan complex which is mainly consisted of elastic microfibrillar components, such as fibrillin-1 and latent transforming growth factor binding proteins, as well as, chaperone molecules, such as clusterin, and cross-linking enzymes, such as LOXL1^[10].

A variety of epithelial, endothelial and mesenchymal cells may be associated with impaired synthesis of the extracellular fibrillar material in intra- and extraocular sites. Intraocular material seems to be produced mainly in the pre-equatorial lens epithelium, the nonpigmented ciliary epithelium and the iris pigment epithelium, and secondarily in the corneal endothelium, the trabecular endothelium and by almost all cell types of the iris stroma^[11]. Extraocular PEX material has been detected by electron microscope in connective tissue of visceral organs and in close proximity to fibroblasts, smooth and striated muscle cells, as well as, heart muscle cells^[12,13]. These types of cells are probably involved in its production throughout the body. The fibrillar material shows ultrastructural and immunohistochemical similarities in both intra- and extraocular sites.

Although there is no clear-cut evidence that these deposits would cause degeneration of the extraocular tissues, they have been associated with cardiovascular and cerebrovascular morbidity. However, the clinical significance of the PEX-related systemic disorders remains controversial, as published results are inconsistent.

Studies implying a relationship between PEX syndrome and cardiovascular disease are mentioned below, along with others not supporting such a relationship.

HEART DISEASES

In Australia, the Blue Mountains Eye Study proposed that a history of angina, hypertension or a combined history of angina, acute myocardial infarction and stroke are significantly associated with the presence of PEX syndrome after multivariate adjustment including age, sex, glaucoma and vascular risk factors. This was attributed to the effect of elastosis in the vessel wall^[14]. Citirik *et al*^[15] found a significantly higher prevalence of PEX in 50 patients with coronary artery disease (CAD) proven by angiography than in healthy controls, and a higher prevalence of CAD in PEX individuals. PEX has been positively associated with presence of CAD among a large cohort of patients scheduled for cataract surgery^[16,17]. More recently, French *et al*^[18] reported significant associations of PEX and PEX glaucoma with a variety of cardiovascular disorders, including various stages of ischemic heart disease, cardiomyopathy and aortic aneurysm. Moreover, subclinical myocardial ischemia, by tissue Doppler echocardiography, has been found in PEX patients^[19].

The possibility of an association between PEX and asymptomatic myocardial diastolic dysfunction (an important cause of heart failure), as assessed by two-dimensional echocardiography and pulsed Doppler echocardiography, has been suggested^[20]. In addition, a higher prevalence of heart failure has been described in PEX

individuals^[21].

Although there is convincing evidence that PEX syndrome is related to cardiovascular disorders, no significant relationship between PEX and CAD, aortic aneurysm or peripheral artery disease was reported by Emiroglu *et al*^[22]. In the same line, arterial hypertension, ischemic heart disease, cerebrovascular disease and prevalence of diabetes mellitus did not differ between patients with or without PEX^[23-27]. Of note, a higher prevalence of arrhythmia has been found in PEX individuals^[23]. Also, a study by Tarkkanen *et al*^[28] failed to show any significant difference in the frequency of hypertension or ischemic heart disease between patients with primary open-angle glaucoma (POAG) and PEX glaucoma, while the latter had a lower frequency of diabetes mellitus. Moreover, in the Thessaloniki Eye Study, no association was found between PEX and the history of specific or any systemic disease (self-reported history of hypertension, diabetes, cardiovascular disease, migraine, heart attack, coronary artery bypass, vascular surgery)^[29]. Avsar *et al*^[30] found no significant differences in time domain heart rate variability parameters (a measure of cardiac autonomic function) between patients with PEX syndrome and control subjects. Furthermore, several studies failed to demonstrate an association between PEX deposits and increased cardiovascular, cerebrovascular or total mortality^[31-35].

VASCULAR AND CIRCULATION DISTURBANCES

Major manifestations of cardiovascular diseases such as a decreased blood flow and ischemia have frequently been documented in PEX syndrome. Deposition of PEX material within the vasculature with subsequent increases in vascular resistance and decreases in blood flow, vascular dysregulation and altered parasympathetic vascular control may be implicated in the pathogenesis of cardiovascular disorders in PEX subjects. Moreover, local ischemia and atherosclerosis have been correlated with elastosis in different tissues^[36,37].

Increased aortic stiffness in PEX patients, which may be at least partially responsible for the increased incidence of CAD in this patient group has been described^[38]. In addition, using the ultrasound wall tracking system Visontai *et al*^[39] reported a lower distensibility and higher rigidity in the common carotid artery, as well as, altered parasympathetic vascular control connected to increased plasma homocysteine level in PEX/PEX glaucoma than the control group. Similar results were drawn by other studies showing lower myocardial peak systolic tissue Doppler imaging velocities and increased carotid intima-media thickness in patients with PEX syndrome when compared to controls. On the contrary, PEX and carotid plaque measurements were weakly correlated^[40]. An impairment of parasympathetic cardiovascular regulation, baroreflex sensitivity and pulse wave velocity has also been described in PEX patients^[41]. Arterial stiffening is an indicator of increased cardiovascular disease risk and, likewise, decreased baroreflex sensitivity has been

described in hypertension, heart failure, myocardial infarction and metabolic syndrome. Lower cutaneous capillary blood flow and altered response to cold and warmth, without any change of plasma endothelin-1 concentration was also demonstrated^[42]. Furthermore, Kőz *et al*^[43] found high levels of coronary risk markers such as lipoprotein (a), apolipoprotein A, homocysteine, as well as, impaired brachial artery dilation and increased carotid intima-media thickness in PEX patients. In a study by Praveen *et al*^[25] PEX subjects had a significantly lower ankle brachial index as compared to controls, suggestive of PEX as a possible risk factor for peripheral vascular disease.

Ocular vascular and blood flow abnormalities

Dayanir *et al*^[44] concluded that PEX decreases ophthalmic artery blood flow velocities and increases vascular resistance. Similar conclusions were drawn by another study where PEX patients had decreased blood flow velocities in the central retinal and the short posterior ciliary arteries and increased vascular resistance in the ophthalmic and central retinal arteries^[45]. Reduced blood flow in choroid, optic nerve head and peripapillary retina of the PEX affected eye has also been found^[46,47]. Moreover, Galassi *et al*^[48] using color Doppler imaging found a decrease in ocular perfusion pressure and deterioration of retrobulbar haemodynamics in PEX glaucoma patients as compared to primary open-angle glaucoma patients and healthy controls. Several studies have demonstrated anterior-chamber hypoxia and iris vasculopathy (narrowing, occlusion, neovascularization) in PEX patients^[49-52]. PEX as a potential risk factor for central retinal vein occlusion has also been proposed^[53,54]. In support of the above, Cursiefen *et al*^[55] found that PEX was significantly more common in eyes enucleated secondary to central retinal vein occlusion as compared to age-matched eyes enucleated for an intraocular tumor; however, morphological evidence of a PEX associated vasculopathy of the central retinal vessels explaining this association was not shown. Endothelin-1, a potent vasoconstrictor which could contribute to the obliterative vasculopathy seems to be increased in the aqueous humor of PEX eyes^[56].

Cerebral vascular and blood flow abnormalities

A high frequency of PEX syndrome has been reported in patients with transient ischemic attacks^[57-59]. A significantly higher prevalence of magnetic resonance images-defined white matter hyperintensities (ischemic changes) in patients with a clinical diagnosis of PEX with or without glaucoma *vs* control subjects, has also been documented^[60]. Studies have indicated that the blood flow velocities of the middle cerebral artery were decreased in patients with PEX and PEX glaucoma^[61,62] and there was a decrease in regional brain perfusion in PEX patients^[63].

In addition, chronic cerebral diseases such as senile dementia, cerebral atrophy and chronic cerebral ischemia were more common in patients with PEX glaucoma than in those with POAG. The same study showed that patients with PEX glaucoma had higher probability of de-

veloping acute cerebrovascular disease than patients with POAG^[31]. Alzheimer's disease has also been correlated to PEX syndrome in several, though not all studies^[64-67].

Systemic arterial endothelial dysfunction

Arterial endothelial dysfunction is an independent predictor of future cardiovascular events. Vascular endothelium has a major role in the control of blood flow by releasing factors which may act either to contract the vascular smooth muscle, such as endothelin-1, or to relax it, such as nitric oxide. Atalar *et al*^[68] found an impaired endothelial function in the brachial artery of patients with PEX syndrome, as assessed by vascular response to reactive hyperemia and sublingual nitroglycerin using high-resolution ultrasound. Endothelial dysfunction was attributed to the pseudoexfoliative fibrillar accumulation in the vessel wall. In the same line, endothelial dysfunction of the brachial artery was described in PEX subjects^[69].

A major theory of atherosclerosis is that lesions result from an excessive fibroproliferative response to various forms of insult to the endothelium and smooth muscle of the vascular wall^[70]. Endothelial exfoliation has been defined as thin, friable, mobile and translucent tissue, loosely adherent to the vascular wall^[71] that may play a functional role in thrombus formation^[72].

Nevertheless, other studies failed to demonstrate a correlation between PEX and endothelial damage, as biomarkers levels of endothelial injury (von Willebrand antigen, E-selectin, P-selectin and high sensitivity C-reactive protein) did not differ in blood plasma of patients with PEX *vs* controls^[73,74].

Elevated homocysteine

Homocysteine is an independent risk factor for cardiovascular disease. It is associated with vascular injury and, thus, increased risk for stroke, CAD and venous thrombosis. Possible mechanisms of action include endothelial dysfunction, platelet aggregation and perturbation of clotting factors. In addition, alteration of the extracellular matrix of several tissues (mainly vessels), elastolysis and oxidative stress may be implicated.

Hyperhomocysteinemia has been suggested as a possible cause for increased vascular risk because of the potential to trigger the abnormal matrix accumulation in PEX patients. High levels of plasma homocysteine have been found in patients with PEX syndrome and PEX glaucoma^[75-84]. Homocysteine concentration has been found to be elevated^[83] or unaffected^[77] in aqueous humor of patients with PEX glaucoma, while increased in PEX glaucoma patients' tears^[84]. Vitamins B6, B12 and folate, which are involved in homocysteine metabolism and negatively correlated with total plasma homocysteine levels, have been reported to be decreased in PEX glaucoma patients^[85], though not differing between PEX and control groups in another study^[77]. On the contrary, Turacli *et al*^[86] did not confirm the relationship between plasma homocysteine and PEX syndrome. Hyperhomocysteinemia has also been implicated in the decrease of both LOX activity and expression in vascular endothelial

cells^[87]. LOX downregulation has been associated with endothelial dysfunction, characteristic of earlier stages of the atherosclerotic process^[88]. A possible association between SNPs in the *LOXL1* gene (which is linked with PEX syndrome) and spontaneous cervical artery dissection has also been proposed^[89].

Arterial hypertension

It is known that hypertension is a major risk factor for stroke, myocardial infarction, heart failure, aneurysms of the arteries (*e.g.*, aortic aneurysm), peripheral arterial disease and chronic kidney disease. At least two studies have demonstrated a higher rate of arterial hypertension in patients with PEX^[14,90]. Endothelial damage, impairment of the parasympathetic vascular regulation and elastosis have been implicated. Renal artery stenosis with subsequent arterial hypertension has also been reported^[91]. However, reports are conflicting and no clear association has yet been proven, as other studies failed to demonstrate any significant relationship between PEX and arterial hypertension^[15,16,17,23-26,61,92], or found arterial hypertension to be less common in PEX subjects^[28,93,94].

Aortic aneurysm

Impairment in systemic macro- and microcirculation in PEX patients has been suggested. Abdominal aortic aneurysms have been attributed to atherosclerosis, though other factors are involved in their formation. An association between aneurysms of the abdominal aorta and PEX syndrome has been proposed. Histopathological examination of aortic-wall samples from patients with ocular PEX syndrome revealed accumulation of focal PEX deposits in the adventitial and subendothelial connective tissue, pronounced fibrosis, and elastosis of the tunica intima^[95]. Abdominal aorta aneurysm was observed with a higher frequency in PEX patients than in control group^[91,96], although, other studies failed to demonstrate any significant association^[97,98].

OTHER COMMON PATHOGENETIC SIGNS

Apart from epidemiologic studies and the presence of PEX deposits on vessel wall, a possible relation between PEX material and cardiovascular disease may be supported by similar features in their pathogenesis. In addition to vascular endothelial dysfunction, hyperhomocysteinemia and blood flow changes mentioned above, disorders of the extracellular matrix by growth factors, matrix metalloproteinases, cytokines and altered enzymic action constitute part of atherosclerosis^[99] and PEX fibrilopathy process. Altintas *et al*^[100] demonstrated higher serum antiphospholipid antibodies (a risk factor for cardiovascular and cerebrovascular disease) in patients with PEX and PEX glaucoma than in healthy controls and in patients with POAG. In support of the above, serum asymmetric dimethyl arginine and YKL-40 levels (both independent cardiovascular risk factors) have been found higher in

PEX patients than those of the control group^[101,102].

Atherosclerosis is associated with a number of oxidative events like low density lipoproteins oxidation, production of intracellular reactive oxygen species (ROS) and reactive nitrogen species (RNS), as well as, endothelial dysfunction and plaque disruption^[103]. The oxidative-antioxidative balance is disturbed in patients with PEX syndrome as supported by reduced levels of antioxidants such as ascorbic acid, glutathione, trace elements, antioxidant enzymes in aqueous humor and serum and increased levels of oxidants such as hydrogen peroxide or nitric oxide, as well as, oxidative stress markers^[104].

Inflammation plays a major role in all phases of atherosclerosis. Inflammatory cells like macrophages and lymphocytes both migrate from the blood and multiply within the atherosclerotic plaques. Activation of these cells leads to lytic enzymes, cytokines, chemokines and growth factors release that induce further damage^[105]. Stress-induced, temporally restricted subclinical inflammation in anterior segment tissues is detected during the early stages of the fibrotic PEX process^[10]. Moreover, inflammatory markers such as alpha-1 antitrypsin, Interleukin-6, high-sensitivity C-reactive protein and Tumor Necrosis factor alpha have been reported to be increased in PEX subjects^[106-108].

CONCLUSION

Although more data is still required, an increased incidence of cardiovascular disorders in PEX patients and several common features in their pathogenesis suggest that PEX may be an independent risk factor for cardiovascular disease or it may occur as part of a systemic disorder with cardiovascular implications. The pathogenesis of PEX glaucoma and CAD in PEX patients may reflect different manifestations of the same process. Patients with PEX syndrome should be informed and examined frequently as cardiovascular risk may be present throughout.

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Management of renal artery stenosis: What does the experimental evidence tell us?

Mohammed Al-Suraih, Joseph Peter Grande

Mohammed Al-Suraih, Joseph Peter Grande, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55905, United States

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Correspondence to: Joseph Peter Grande, MD, PhD, Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, United States. grande.joseph@mayo.edu

Telephone: +1-507-2669356 Fax: +1-507-2661163

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Abstract

Optimal management of patients with renal artery stenosis (RAS) is a subject of considerable controversy. There is incontrovertible evidence that renal artery stenosis has profound effects on the heart and cardiovascular system in addition to the kidney. Recent evidence indicates that restoration of blood flow alone does not improve renal or cardiovascular outcomes in patients with renal artery stenosis. A number of human and experimental studies have documented the clinical, hemodynamic, and histopathologic features in renal artery stenosis. New approaches to the treatment of renovascular hypertension due to RAS depend on better understanding of basic mechanisms underlying the development of chronic renal disease in these patients. Several groups have employed the two kidney one clip model of renovascular hypertension to define basic signaling mechanisms responsible for the development of chronic renal disease. Recent studies have underscored the importance of inflammation in the development and progression of renal damage in renal artery stenosis. In particular, interactions between the renin-angiotensin system, oxidative stress, and inflammation appear to play a critical role in this process. In

this overview, results of recent studies to define basic pathways responsible for renal disease progression will be highlighted. These studies may provide the rationale for novel therapeutic approaches to treat patients with renovascular hypertension.

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Key words: Renovascular hypertension; CCL2; CCR2; Kidney; Inflammation; Atrophy

Core tip: Renovascular hypertension is a common public health problem, particularly in older patients with underlying atherosclerotic vascular disease. Recent studies have shown that restoration of blood flow in these patients fails to improve renal function or survival. Recent studies to define basic mechanisms underlying the development of chronic renal disease in renin angiotensin system (RAS) have shown that pro-inflammatory pathways may play a critical role in this process. Therapeutic approaches that target inflammatory pathways may provide the basis for novel and more effective treatments for patients with RAS.

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RENOVASCULAR HYPERTENSION IS AN IMPORTANT CAUSE OF SECONDARY HYPERTENSION

It is well recognized that hypertension is a major public health problem. The prevalence of hypertension is 29% in the United States; an additional 28% of adults have

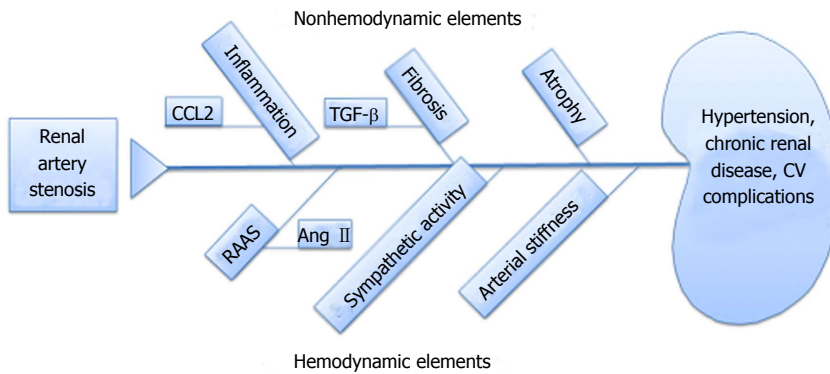


Figure 1 Summary of hemodynamic and non-hemodynamic pathways responsible for development of chronic renal damage in renal artery stenosis.

“prehypertension”^[1]. Although the most common form of hypertension is “essential” hypertension, there is increasing recognition of secondary forms of hypertension that contribute to morbidity and mortality in patients with hypertension. Many of these cases have been identified through use of imaging modalities to assess patency of the coronary arteries. The prevalence of renovascular hypertension (RVH) is 7% in patients over 65 years of age^[2]. In patients with coronary artery disease or aortoiliac disease, the prevalence of RVH is as high as 50%^[3-5]. From 1991-1997, the annualized incidence of RVH as a cause of end stage renal disease increased by 12.4% per year, a larger increase than other causes of end stage renal disease^[6]. Atherosclerosis is the most common etiology underlying RVH in this population^[7-9]. In addition to chronic renal disease, atherosclerotic RVH contributes to cardiac morbidity and mortality^[10]. For example, recent studies have shown that the overall 4-year survival of patients undergoing cardiac catheterization was 86% in patients without RAS but only 65% in those with RAS^[11]. The extent of RAS also predicts survival, with 4-year survival of 89% in patients with RAS < 75% luminal occlusion, but only 57% in those with > 75% luminal occlusion^[10,11]. Optimal treatment of these patients require the development of animal models to elucidate mechanisms of renal and cardiovascular disease progression.

ANIMAL MODELS OF RVH

The two kidney 1 clip (2K1C) model of renovascular hypertension, developed by Goldblatt, has been extensively employed to understand the pathogenesis of renovascular hypertension^[12]. In his original model, dogs subjected to renal artery stenosis developed malignant hypertension, which caused extensive damage to the contralateral kidney. More recently, this model has been extended to other species, including mice, rats, and pigs^[13-19]. In general, these animals do not develop malignant hypertension, and may thereby more accurately model human renal artery stenosis. In these animals, the stenotic kidney develops progressive atrophy, whereas the contralateral kidney develops hypertrophy but without major histopathologic alterations^[14].

This model has allowed investigators to study the interrelationships between hemodynamic factors and non-hemodynamic factors responsible for the development

of cardiovascular and renal disease (Figure 1). Hemodynamic factors include vasoactive effects mediated by activation of the renin-angiotensin-aldosterone system, increased sympathetic nervous activity, and increased arterial stiffness. Non-hemodynamic factors include the signaling pathways triggered by renal parenchymal cells and infiltrating inflammatory cells in the development and progression of renal and cardiovascular disease, and include chemokines, reactive oxygen species, and transforming growth factor β (TGF β).

DEVELOPMENT OF CHRONIC RENAL DISEASE IN RAS: WHAT DOES THE EXPERIMENTAL EVIDENCE TELL US?

Most studies have focused on the role of renal hypoperfusion and subsequent hypoxia on the development of chronic renal damage in the stenotic kidney. It is well recognized that reduced blood flow leads to intra-renal activation of the renin-angiotensin system, leading to elevated plasma levels of angiotensin II, a potent vasoconstrictor, and the development of systemic hypertension. However, several recent observations have called this paradigm into question. Recent imaging studies to assess renal oxygenation have suggested that the stenotic kidney is not hypoxic. It is recognized that the kidney receives far more blood than needed to support basic metabolic demands—indeed, renal tissue requires less than 10% of normal blood flow to support basic metabolic needs^[20]. Furthermore, the kidney has the capacity to adapt to significant reduction in the diameter of renal artery with preservation of renal oxygenation^[21]. In both human and experimental models, it appears that systemic activation of the renin-angiotensin system is transient, and that progression of renal and cardiovascular disease can occur without persistent elevation of plasma angiotensin II levels^[22]. These observations have prompted investigations into basic signaling pathways triggered by renal artery stenosis that may be responsible for maintenance of systemic hypertension and the development of chronic renal disease.

Although plasma angiotensin II levels may not remain elevated as cardiac and renal damage progress in renal artery stenosis, there is evidence for persistent activation of the intra-renal renin-angiotensin system. The

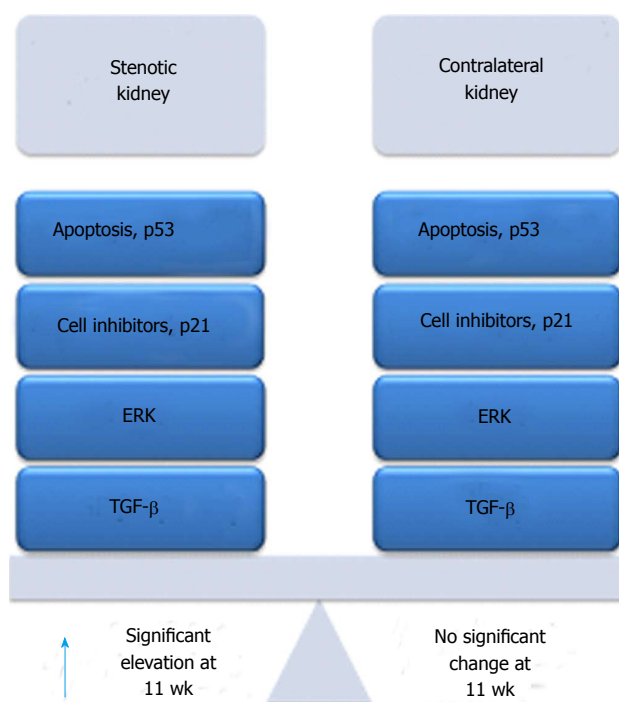


Figure 2 Mediators persistently induced in the stenotic kidney and transiently induced in the contralateral kidney in murine renal artery stenosis.

kidney can produce all elements needed to completely activate the renin-angiotensin system, including renin, angiotensinogen, angiotensin converting enzyme, and angiotensin type 1 and type 2 receptor^[23-25]. In the kidney, renin is expressed primarily by the juxtaglomerular cells. Angiotensinogen is expressed in proximal tubular epithelial cells and is secreted into tubular lumens. Angiotensin I is converted to Angiotensin II through action of ACE located on the apical brush border of tubular epithelium. We have shown that renal expression of Ren1 in the stenotic, but not contralateral, kidney persists in renal artery stenosis^[26]. Based on these considerations, we embarked on a series of studies to compare signaling pathways activated in the stenotic and contralateral kidneys during the development and progression of renal damage in renal artery stenosis. A summary of our findings is highlighted in Figure 2.

In our initial studies, we correlated histopathologic alterations in the stenotic and contralateral kidneys at 2, 5, and 11 wk following renal artery stenosis surgery with signaling pathways that govern cell cycle regulation (cyclins D, E, A, and B; p21; p27), proliferation (PCNA, ERK, p38 MAPK), fibrosis (TGF-β; Smad2, Smad3, Smad4), and inflammation (MCP-1)^[14]. The stenotic kidney showed progressive tubular atrophy, which was associated with interstitial fibrosis and inflammation, which closely recapitulates the histopathologic alterations observed in humans with advanced renal artery stenosis^[27]. The contralateral kidney underwent compensatory enlargement, which was at least in part through hyperplasia. Compensatory enlargement in the contralateral kidney occurred in the absence of significant histopathologic alterations. We found that signaling pathways associated

with cell cycle regulation, inflammation, and fibrosis were activated in both kidneys following induction of renal artery stenosis. However, these pathways were transiently activated in the contralateral kidney, returning to baseline levels by 11 wk, whereas they were progressively and persistently activated in the stenotic kidney.

A critical role for the p38 MAPK pathway in the development of renal atrophy was established in studies using the biochemical inhibitor SB203580^[28]. The development of renal atrophy in the stenotic kidney was significantly decreased in mice treated with SB203580 at the time of renal artery stenosis surgery. Decreased atrophy was associated with reduced interstitial inflammatory infiltrates and decreased fibrosis. The p38 MAPK inhibitor had no significant effect on blood pressure or on plasma renin activity. Of note, treatment of mice with the ERK inhibitor U0126 did not prevent the development of renal atrophy, interstitial fibrosis, and interstitial inflammation (unpublished data).

In our previous studies, we demonstrated that TGF-β and its receptors (RI and RII) are persistently induced in the stenotic kidney of mice subjected to RAS. TGF-β has been implicated as a critical mediator of cell cycle regulation, inflammation, and fibrosis in other model systems^[14,29-31]. The TGF-β signaling pathway interacts with a number of other signaling pathways, including the renin-angiotensin system and the MAPK pathways. TGF-β mediates fibrosis through interactions with Smad 2, Smad3, and Smad4. Although TGF-β knockout mice have high embryonic lethality and develop a systemic inflammatory syndrome shortly after birth, Smad3 knockout mice are viable and exhibit defects in TGF-β signaling^[32].

We found that the stenotic kidneys of Smad3 knockout mice are almost completely protected from the development of interstitial fibrosis, tubular atrophy, and interstitial inflammation, despite an elevation of plasma renin activity and a reduction in blood flow of over 70%^[22]. In an acute ischemia-reperfusion model, we showed that the kidneys of Smad3 knockout mice were resistant to the development of acute injury^[33]. A similar protective effect has been observed in Smad3 mice subjected to unilateral ureteric obstruction.

Although we have shown that interruption of the p38 MAPK or Smad3-TGF-β signaling pathways prevent the development of renal atrophy, it is not clear how renal damage is initiated in this model. For this reason, we have conducted a series of studies to better understand the early signaling events and to correlate these with histopathologic alterations during the development of chronic renal disease in this model^[26]. At 3 d following renal artery stenosis surgery, the stenotic kidney shows minimal histopathologic alterations. In particular, there is no evidence of acute injury to tubular epithelial cells, no significant interstitial fibrosis, tubular atrophy, or interstitial inflammation. Despite the normal appearance of the stenotic kidney, the tubular epithelial cells express markers of oxidative stress. It is recognized that the kidney expresses all components of the NADPH oxidase system^[34] and that Ang II promotes ROS generation through activation of

this membrane bound complex^[34]. However, most studies of the interaction between Ang II activation and ROS generation have focused on later time points, after the initiation and development of chronic renal injury.

Influx of inflammatory cells, predominantly macrophages and lymphocytes, is first seen at 7 d following RAS surgery, a time point at which the kidney begins to develop tubular atrophy^[26]. Influx of inflammatory cells is associated with induction of CCL2 (MCP-1) a potent chemoattractant protein. The relevance of this observation is underscored by studies demonstrating that increased production of CCL2 is associated with the influx of inflammatory cells in human renal artery stenosis^[35,36] and that the development of chronic renal disease in RAS is associated with the influx of macrophages and T cells. Recent studies have suggested that signaling through CCL2 may play a critical role in the development and progression of both renal and cardiovascular disease in renal artery stenosis and other cardiovascular and renal diseases^[37-39]. Both renal parenchymal cells and infiltrating inflammatory cells express CCR2, the primary receptor for CCL2. Through activation of this pathway, infiltrating inflammatory cells are capable of generating ROS and a number of chemokines which promote renal fibrosis.

We have observed increased expression of CCL2 at 3 d, prior to the influx of inflammatory cells, suggesting that renal parenchymal cells may be the source of this chemotactic chemokine^[26]. Additional studies are required to verify this observation. It is not yet known whether blockade of CCL2-CCR2 signaling will prevent the development and/or progression of chronic renal disease in RAS.

MANAGEMENT OF RENAL ARTERY STENOSIS

Until recently, management of RAS was predicated on restoration of blood flow to the stenotic kidney. It was thought that this intervention would decrease local and systemic activation of the renin-angiotensin system, thereby restoring normal blood pressure and reducing both renal and cardiovascular morbidity and mortality. Recent studies have clearly demonstrated that restoration of blood flow through stenting fails to improve renal or cardiovascular outcomes, compared to medical therapy^[40]. The mainstay of medical therapy involves blood pressure control, through use of angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and/or other agents to reduce blood pressure^[41]. There are concerns that aggressive blood pressure reduction may exacerbate damage to the stenotic kidney, although reduction of blood pressure is thought to improve overall renal function by protecting the contralateral kidney. Unfortunately, patients treated with medical therapy are still at risk for the development of progressive chronic renal disease, which in itself is a risk factor for the development of cardiovascular events. Recent studies have raised the possibility that therapies directed towards modulating the

inflammatory response to chronic renal injury may have a role in management of patients with renal artery stenosis.

CONCLUSION

Optimal management of patients with RAS is limited by our lack of understanding of the events leading to the development of chronic renal damage in the stenotic kidney, and how these events contribute to cardiovascular morbidity and mortality. Inhibitors of P38 MAPK and of Smad3 signaling have been shown to prevent the development of chronic renal damage in experimental RAS. In addition to concerns regarding adverse effects of currently available compounds, there is no evidence that these agents can prevent the progression of chronic renal damage once clinical manifestations of renal artery stenosis become apparent. Similarly, human trials of antioxidant therapies to arrest the progression of systemic inflammatory conditions including atherosclerosis have been disappointing. Our recent observations, that generation of CCL2 and expression of CCR2 is an early event in RAS—an event which precedes the influx of inflammatory cells—merit additional study. In particular, it is not known whether abrogation of CCL2-CCR2 signaling will prevent the development of chronic renal disease in RAS or will arrest the progression of chronic renal disease once the disease becomes clinically apparent. Studies to address these important issues may provide the basis for changing the paradigm for treatment of renal artery stenosis from one that emphasizes restoration of renal blood flow to one that focuses on treatment of the inflammatory response to renal artery stenosis.

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Long term negative pressure ventilation: Rescue for the failing fontan?

Shriprasad R Deshpande, Kevin O Maher

Shriprasad R Deshpande, Kevin O Maher, Division of Pediatric Cardiology, Emory University, Children's Healthcare of Atlanta, Atlanta, GA 30322, United States

Author contributions: Both authors contributed to this work.

Correspondence to: Shriprasad R Deshpande, MD, MS, Division of Pediatric Cardiology, Emory University, Children's Healthcare of Atlanta, 1405 Clifton Rd NE, Atlanta, GA 30322, United States. deshpandes@kidsheart.com

Telephone: +1-404-6947739 Fax: +1-770-4889480

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we review the pathophysiology of failing Fontan, current therapies and propose a novel way of treating the failing Fontan by utilizing negative pressure ventilation to reverse some of the maladaptive changes. This is a hypothesis paper. We think, the ideas central to the manuscript are worth bringing out for intellectual discussion and wider testing.

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Abstract

Current treatment strategies for single ventricle patients include non-intervention strategy, surgical palliation or primary transplantation. Surgical palliation includes a staged operative course culminating in the Fontan operation. With progress in surgical techniques, the survival has been improving. However, almost all of these Fontan patients will demonstrate pathophysiologic changes that ultimately constitute "Fontan failure physiology". This article reviews the pathophysiologic changes, current approach to management of these patients and proposes a novel way of reversing some of the pathophysiologic changes by utilization of negative pressure ventilation.

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Key words: Fontan; Single ventricle physiology; Negative pressure ventilation; Cardiorespiratory interactions; Congenital heart disease

Core tip: In the current surgical era for congenital heart disease, palliation of single ventricle patients has become standard of care. However, pathophysiologic failure after the third stage of palliation (Fontan) is commonplace, with very few therapeutic options. Failing Fontan physiology is a management challenge. Herein,

INTRODUCTION

The Fontan pathway is a palliative pathway for single ventricle patients. This pathway allows us to utilize the single ventricle as a systemic pumping chamber and create separation between the pulmonary and systemic circuits thereby allowing sustenance of life. We have therefore dramatically altered the natural history of these congenital heart problems.

Over the last two decades, with significant improvement in the surgical and perioperative technologies, the mortality of complicated cardiac surgeries such as the Fontan procedure has been reduced^[1-3]. However, as the current Fontan population becomes older, we are facing a new challenge of managing failing Fontan circulations. Currently we have very limited options for management of the failing Fontan physiology^[4,5]. This paper proposes new modality for management of these complex patients and the clinico-pathologic evidence for its use.

THE FAILING FONTAN

Fontan or total cavo-pulmonary connection is a staged surgical palliation of functional single ventricle. It allows

us to designate the single ventricle (or the dominant ventricle) as the systemic ventricle. The other essential part of this pathway, then, is to establish source of pulmonary blood flow without a designated “pulmonary” ventricle. At completion, this constitutes a staged connection of the superior vena cava to the pulmonary artery (Glenn procedure) followed by connection of the inferior vena cava to the pulmonary artery (Fontan procedure).

In the current era, this inferior vena cava to the pulmonary artery connection is made by using either an intra-atrial baffle (lateral tunnel) or by using an extracardiac conduit. After completion of this stage of repair, the systemic venous return is channeled appropriately to the pulmonary artery for oxygenation, while the pulmonary veins return to the common atrium, to be ejected out of the single systemic ventricle. Thus, circulation in series is established. This allows, in theory, for fully saturated blood to be pumped out to the systemic circulation. In practice, saturations are around 92% to 94% early postoperatively, with small arteriovenous malformations and coronary sinus blood flow contributing to the lower saturation^[6]. However, as the patients get older, there is a gradual decline in the oxygen saturations due to various factors. Progressive desaturation is only one of the problems of Fontan in later years. Lack of the pulmonary ventricle eventually leads to multiple problems related to the hemodynamics of failing Fontan circuit. Main reasons for late mortality are related to arrhythmias, thromboembolism and protein losing enteropathy^[7]. Other manifestations of the failing Fontan circuit include systemic venous congestion, hepatic dysfunction, coagulopathy, plastic bronchitis, progressive cardiac failure and cardiac cachexia. These are major causes of morbidity and mortality in Fontan patients^[4,5,8].

Along with the above, there is progressive decrease in the forward flow of blood to the pulmonary vascular bed, leading to progressive hypoxemia and cyanosis. Development of systemic to pulmonary venous collaterals further contributes to the development of cyanosis^[6].

There are limited medical and surgical options for management of these patients^[4,9,10]. For some patients who meet the eligibility criteria including a low pulmonary vascular resistance, heart transplantation is an option. The early outcomes of heart transplantations in patients with failed Fontan are slightly worse compared to patients with cardiomyopathies or other congenital heart diseases^[11,12]. Heart transplantation is therefore a reasonable option in selected group of patients, with organ supply being a significant limiting factor. Patients with classic atriopulmonary connection and incessant arrhythmias or flow obstruction may need conversion to an extracardiac cavo-pulmonary connection^[9]. Other surgical interventions focus on relieving obstructive causes of Fontan failure (e.g., conduit obstruction) or systemic atrioventricular valve replacement for significant regurgitation. As a palliation for high Fontan pressures, creation of a fenestration from the Fontan to the atrium is considered^[13].

Medical management of failing Fontan focuses on treating individual issues^[4,5]. Systemic venous congestion

and volume overload is treated with diuretics. Aggressive diuresis however, can be counterproductive. Anticoagulation, either with anti-platelet agents or coumadin is used in the presence of thrombosis. Myocardial dysfunction manifests itself as both systolic and diastolic dysfunction. Severe myocardial dysfunction may warrant intravenous milrinone therapy. There is limited data to suggest significant benefits occur from using ACE inhibitors or beta-blockers in failing Fontan^[14,15]. Similarly, newer agents such as endothelin receptors antagonists have failed to show impact in Fontan patients. Medical therapy for other complications such as protein losing enteropathy has only had modest success^[16].

As mentioned above, all of these constitute piecemeal approach and none of these strategies address the one of the primary problems, which is, decreased antegrade flow across the Fontan circuit to the pulmonary vascular bed causing the pathophysiology of failure.

HEMODYNAMIC EFFECTS OF NEGATIVE PRESSURE VENTILATION

Negative pressure ventilators were one of the first ventilators developed and served a vital role during the Polio epidemics in the twentieth century. Overtime, positive pressure ventilators have completely replaced them as conventional modes of ventilation. As a result, there are very limited circumstances in contemporary medicine under which negative pressure ventilation negative pressure ventilation (NPV) is being considered^[17,18]. An example would be patients with neuromuscular disorders for long term respiratory support^[17].

Currently, there are some commercially available devices for delivering NPV. Porta- LungTM is a modern version of the iron lung. It is a closed chamber system that delivers effective negative pressure ventilation and has been used for long term ventilatory support. Cuiras[®] ventilator is a shell that is applied over the chest and delivers NPV. This mode applies negative pressure locally over the thorax only and allows for better patient mobility and ease of access. The ventilators that drive these units have also undergone significant improvements over the years, including ability to synchronize breaths with patient initiated breaths as well as with cardiac cycle^[19].

From cardiac and hemodynamic standpoint, NPV has significantly different effects as compared to positive pressure ventilation (PPV). These cardiopulmonary interactions are much more physiologic than those of PPV.

In a normal heart, NPV and by extension, negative intrathoracic pressure leads to reduction in the right ventricular afterload thus augmenting right ventricular function and right ventricular cardiac output. NPV helps maintain lung volumes close to functional residual capacity, which reduces the pulmonary vascular resistance and improves pulmonary blood flow^[20]. In physiologic states as well as in patients after simple cardiac surgery, NPV has been shown to augment cardiac output^[21]. In patients with Glenn or Fontan physiology, where there is depen-

dence on passive diastolic blood flow, NPV directly augments passive blood flow to the lungs by creating a negative thoracic gradient^[22,23]. As a downstream consequence, there is an increase in the pulmonary venous return and cardiac output.

In experimental models and small studies, benefits of NPV in immediate post-operative period have been documented^[24]. Shekerdemian *et al.*^[25,26] have shown hemodynamic benefits of NPV in patients with right ventricular dysfunction in post-operative period^[25,26]. Similarly, augmentation of cardiac output by using NPV, in the immediate post operative period for patients undergoing Fontan procedure has also been documented^[23].

We have recently documented the dramatic application of a NPV in the rescue of a failing Kawashima patient, resulting in successful recovery after failure of all conventional therapies^[27].

All of these applications of NPV in Fontan patients have been for a very short term ; either in the immediate post-operative state or during hemodynamic studies. There has not been an application for long-term use of NPV in cardiac patients as a rescue measure or mode of palliation for these single ventricle patients. We propose such a novel application, based on strong hemodynamic reasoning as outlined above as well as the aforementioned short term application studies.

HYPOTHESIS

Our hypothesis is that long-term use of negative pressure ventilation is an effective mode of rescue for patients with failing Fontan physiology. Our hypothesis extends to suggest that long term use of NPV will: (1) improve antegrade flow to the pulmonary bed across the Fontan circuit (by creating intrathoracic negative pressure). This in turn would lead to: decrease Fontan pressures; decrease hepatic vein wedge pressure thereby decreasing hepatic congestion and improving hepatic function; decrease formation of ascites; and decrease peripheral edema; (2) stabilize and even improve oxygen saturation (better Fontan flow and improved oxygenation); (3) improve cardiac output (based on 21-23); and (4) provide symptomatic improvement as measured by exercise capacity and patient self-assessment scores.

CLINICAL APPLICATION

The proposed method of practical application of this management strategy is as follows. The initial step is appropriate patient selection. Patients who have undergone Fontan procedure and have been classified as failing Fontan patients will be candidates for this therapy. Patients with fixed obstruction that is reversible (such as stenosis of branch pulmonary artery) should be intervened on prior to selection. All patients should get a comprehensive imaging workup, either with echocardiography or an MRI where echocardiography is inadequate.

The recommended method of delivery of NPV is by using a synchronized biphasic cuirass ventilator. Initiation

of NPV should be in hospital setting. This will provide closer monitoring during initiation as well as allow adjustments on ventilator setting, assessment of patient comfort and patient education. A baseline complete metabolic assessment including electrolytes, liver function tests and brain natriuretic peptide (BNP) should be obtained. More invasive monitoring including blood gases (arterial and mixed venous) as well as pulmonary artery pressure should not be mandated, but may be beneficial during initial experience.

Settings on the NPV to be optimized as tolerated. After this short stay, patients should be able to use the NPV at home. Home NPV therapy may be designed with various levels of intensity. The proposed level is about 10 to 12 h of NPV during evening and night hours , thus allowing patients to continue with there daily activities during the day time. For younger patients as much as 16 h of NPV time would be recommended. Recommendations for follow-up include telephone call follow-up every week to address any concerns as well as maintain compliance. Patients will be asked to check weights at home every week.

Follow-up as outpatient should be in two weeks initially, followed by monthly until the care-giver deems appropriate. A repeat complete metabolic panel and BNP should be obtained in 3 mo. Functional status assessment as well as exercise capacity testing should be performed at 6 mo. Continued follow-up to assess improvement in hemodynamics and symptomatology as deemed appropriate by the primary cardiologists should be maintained.

Possible problems related to long-term use of NPV are very minimal and have been described in other settings. Main issues are related to obtaining a good comfortable fit so as to minimize skin contact injury. Patients with upper airway obstruction or significant tracheomalacia are not suitable candidates for NPV and should be excluded^[17,18].

CONCLUSION

Palliation of single ventricle patients has led to increase in long term survival for these complex patients. Current staged surgical palliation concludes with Fontan surgery. However, there are multitudes of problems related to the Fontan circulation that result in significant morbidity and mortality, ultimately resulting in a state of Fontan failure.

As described above, there are limited options for management of a failed Fontan. Here in we propose an innovative use of NPV to augment the Fontan flow and improve the underpinnings of the pathophysiology of Fontan failure.

There is strong experimental and clinical data to suggest that NPV augments the hemodynamics in patients with single ventricle physiology^[21-25]. The ability of the modern negative pressure ventilators to be portable, accessible and effective has provided the opportunity of unique application of these ventilators as a long term therapy for failing Fontan patients.

The authors propose that this strategy will provide a

novel therapy to address a growing problem and provide improved quality of life to this group of patients.

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Risk stratification for ST segment elevation myocardial infarction in the era of primary percutaneous coronary intervention

Richard A Brogan, Christopher J Malkin, Phillip D Batin, Alexander D Simms, James M McLenachan, Christopher P Gale

Richard A Brogan, Christopher P Gale, Leeds Institute of Cardiovascular and Metabolic Medicine, Division of Epidemiology and Biostatistics, University of Leeds, Leeds LS2 9JT, United Kingdom

Richard A Brogan, York and Hull Medical School, University of York, York, YO10 5DD, United Kingdom

Christopher J Malkin, Alexander D Simms, James M McLenachan, Department of Cardiology, Leeds Teaching Hospital NHS Trust, Leeds LS2 9JT, United Kingdom

Phillip D Batin, Department of Cardiology, Pinderfields General Hospital, Mid Yorkshire NHS Trust, Wakefield WF5 9LZ, United Kingdom

Christopher P Gale, Department of Cardiology, York Teaching Hospital NHS Foundation Trust, York YO10 5DD, United Kingdom

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Correspondence to: Richard A Brogan, MB ChB, BSc, MRCP, Leeds Institute of Cardiovascular and Metabolic Medicine, Division of Epidemiology and Biostatistics, University of Leeds, Level 8, Worsely Building, Clarendon Way, Leeds, West Yorkshire, LS2 9JT, United Kingdom. richard.brogan@nhs.net

Telephone: +44-01-133438924 Fax: +44-01-133434877

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techniques in NSTEMI have been demonstrated to improve outcomes however their uptake has been poor perhaps due to questions over their discrimination and concern for application to individuals who may not have been adequately represented in clinical trials. STEMI is perceived to carry sufficient risk to warrant emergency coronary intervention [by primary percutaneous coronary intervention (PPCI)] even if this results in a delay to reperfusion with immediate thrombolysis. Immediate thrombolysis may be as effective in patients presenting early, or at low risk, but physicians are poor at assessing clinical and procedural risks and currently are not required to consider this. Inadequate data on risk stratification in STEMI inhibits the option of immediate fibrinolysis, which may be cost-effective. Currently the mode of reperfusion for STEMI defaults to emergency angiography and percutaneous coronary intervention ignoring alternative strategies. This review article examines the current risk scores and evidence base for risk stratification for STEMI patients. The requirements for an ideal STEMI risk score are discussed.

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Key words: ST segment elevation myocardial infarction; Risk stratification; Primary percutaneous coronary intervention; Harm; Risk scores

Abstract

Acute coronary syndromes presenting with ST elevation are usually treated with emergency reperfusion/revascularisation therapy. In contrast current evidence and national guidelines recommend risk stratification for non ST segment elevation myocardial infarction (NSTEMI) with the decision on revascularisation dependent on perceived clinical risk. Risk stratification for STEMI has no recommendation. Statistical risk scoring

Core tip: Risk stratification is recommended in non ST segment elevation myocardial infarction (NSTEMI) by multiple international cardiology agencies however there is no such recommendation for STEMI. The short term risk of STEMI is perceived to be high and warrant emergency percutaneous coronary intervention rather than pharmacological fibrinolysis. The risk spectrum is wide therefore consideration should be given to developing an optimal reperfusion strategy based on risk of adverse outcome and probability of reperfusion regard-

less of mode of reperfusion.

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INTRODUCTION

Acute coronary syndromes in contemporary cardiology practice

The initial management of acute coronary syndromes (ACS) depends on the presence of ST elevation on the electrocardiogram. In the United Kingdom Primary Percutaneous Coronary Intervention (PPCI) is the recommended treatment for ST segment elevation MI (STEMI). International guidelines recommend formal risk stratification using a validated risk score for all patients presenting with non ST elevation MI (NSTEMI) but not for STEMI.

In this article we review the established risk scores and their limitations. We also examine the need for a risk score for those patients presenting with STEMI.

Risk stratification and risk scores

Risk stratification is defined as “a statistical process to determine detectable characteristics associated with an increased chance of experiencing unwanted outcomes”^[1]. When applied to ACS risk stratification has helped target healthcare resources and guide clinicians as to revascularisation requirement, urgency and method. Risk scores such as the Global Registry of Acute Coronary Events (GRACE) score have shown that of the spectrum of patients with ACS those who presented with STEMI had the highest short-term risk of death. This group also benefitted from rapid reperfusion therapy, an effect confirmed in the GISSI-1 and ISIS-2 trials^[2,3]. Reperfusion treatment was initially limited to systemic thrombolysis (fibrinolysis). However, thrombolysis is associated with a “failure rate” of incomplete coronary reperfusion, which led to the development of mechanical reperfusion methods and the introduction of PPCI programmes^[4].

Within the STEMI population, there is a spectrum of higher and lower risk patients. For example, STEMI presenting with haemodynamic instability or cardiac arrest is associated with a higher risk of mortality^[5,6]. Stratification of risk in STEMI has been more difficult because PPCI has been offered and incorporated into national and international guidelines to all patients without contraindication who present with clinical and electrocardiographic criteria^[7,8]. In contemporary practice it is, therefore, unlikely that a STEMI risk score would impact on decision making, since the pathway is algorithmic once a diagnosis is made. Risk scoring is therefore only used to evaluate

hospital and individual operator performance. An alternative approach would be to use risk scoring in STEMI to target healthcare and refine decision-making such as by offering immediate thrombolysis to low risk patients presenting early and PPCI to other higher risk patients.

Despite progress in pre-hospital care, ambulance logistics, pharmacotherapy and PPCI techniques, STEMI continues to confer a substantial burden of morbidity and mortality and consumes significant healthcare budget. Consequently, optimal reperfusion strategy is a subject of ongoing research interest^[9,10]. When compared to the NSTEMI population there has been little effort to quantify patient risk in STEMI since all randomised controlled trials studying PPCI efficacy offer PPCI as default^[7,8,11].

PPCI when available or immediate fibrinolysis?

Reperfusion is most effective when delivered early. Any delay to reperfusion is associated with an increase in mortality^[12-14]. In the real world patients may experience considerable delays that may negate the benefit of PPCI over immediate fibrinolysis^[15,16]. The National Institute of Health and Care Excellence (NICE) has highlighted the need for further research into very early presentation of STEMI but acknowledges the current evidence in favour of PPCI^[17]. The question of whether early pre-hospital thrombolysis with subsequent coronary angiography and intervention (PCI or CABG) is non-inferior to expert and timely PPCI has been evaluated recently. The Strategic Reperfusion Early after Myocardial Infarction (STREAM) study investigated early fibrinolysis *vs* PPCI. For those with early fibrinolysis with Tenecteplase (TNK) there was a suggestion of outcome equivalence albeit with an increase in intracranial bleeding^[18].

PPCI RISK MODELS FOR DEATH AND BLEEDING IN CONTEMPORARY PRACTICE

The Myocardial Ischaemia National Audit Project (MINAP) is a United Kingdom national registry database of all acute coronary syndromes. The MINAP database was established in 1999 to examine the quality of management of acute myocardial infarction (AMI) in England and Wales and to meet the audit requirements of the national service framework for coronary heart disease^[19,20]. Risk scores have been constructed based on trial data and statistical modelling using databases such as MINAP as bench markers for validity. The other major risk scores are summarised in the table below (Table 1).

The risk scores outlined have demonstrated some ability to predict survival. However, whilst their use has been recommended by international guidelines, their uptake by the clinical community has been poor. There are several reasons for this: The GRACE score is the most widely used but lacks point of care convenience whilst the TIMI score has this functionality but is less discrimi-

Table 1 Summary of major risk scores utilised in percutaneous coronary intervention

Risk score	Type	Population	No of patients	Outcomes	No of variables	Validation	c- statistic	Ref.
GRACE	Clinical	NSTEMI, STEMI	85771	In hospital and 6 mo mortality (8.6% and 12.9%)	7	FAST-AMI	0.8 and 0.8	[21]
GRACE - 2	Clinical	NSTEMI, STEMI	32037	1 and 3 yr mortality	8	FAST AMI	0.82 and 0.82	[22]
GUSTO -1	Clinical	STEMI	41021	30 d to 1 yr mortality (2.9%)	7	MINAP	0.8 at 30 d 0.75 at 1 yr	[21,23]
SRI	Clinical	STEMI	100686	30 d mortality	3	In time II/MINAP	0.79	[21,24]
TIMI	Clinical	STEMI	14114	30 d mortality	10	External with TIMI-9 trial	0.746	[25]
CADILLAC	Clinical	STEMI	2082	1 yr mortality	7	Stent- PAMI (900 patients, internal)	0.78	[26]
APEX - AMI	Clinical	STEMI	5745	90 d mortality	7	Internal (no external)	0.81	[27]
EMMACE	Clinical	All MI	100686	30 d mortality	3	Internal	0.78	[28]
SYNTAX	Angiographic	NSTEMI CSA		5 yr mortality	n/a	LEADERS trial	0.62	[29-33]
Clinical SYNTAX	Clinical and angiographic	NSTEMI CSA	512	5 yr mortality	Syntax score and modified ACEF score	LEADERS trial	0.69	[29]
EURO Heart	Clinical and angiographic	ACS and STEMI	23032	In-hospital mortality	16	Internal	0.89	[34]
MINAP (reference)				30 d to 1 yr mortality (5.0%)				

GRACE: Global registry of acute coronary events; FAST-AMI: French registry of Acute ST-elevation and non-ST elevation MI; GUSTO: Global utilisation of streptokinase and tissue plasminogen activator (TPA) for Occluded coronary arteries; SRI: Simple risk index; TIMI: Thrombolysis in acute myocardial infarction; CADILLAC: Controlled abciximab and device investigation to lower late angioplasty complications trial; APEX: Ami assessment of pexelizumab in acute myocardial infarction trial; EMMACE: Evaluation of methods and management of acute coronary events; SYNTAX: Synergy between pci with taxus and cardiac surgery trial; CSA: Chronic stable angina; ACEF: Age, creatinine, ejection fraction score; LEADERS: Limus eluted from a durable versus erodable stent coating trial.

natory. The SRI, GUSTO and CADILLAC scores are seldom used in clinical practice and external validation is limited. Perhaps the major limitation of all these scores is that myocardial infarction is not always sub-divided into NSTEMI or STEMI. Finally some of the scores (including the TIMI risk score) are based on data derived from a pre-PPCI era or are based on angiographic findings that can not be known at the time of patient presentation.

However, the single dominant reason risk scores are rarely used for STEMI patients is the assumption that all patients presenting with STEMI are at high risk. Furthermore current evidence and international guidelines encourage the rapid diagnosis and treatment with no requirement for risk stratification. The fallibility of risk scores for STEMI is compounded by the issue of timing of data availability for data for a risk score calculation the emergency management of STEMI should not be delayed for the purpose of completing a range of risk parameters which may not be immediately available. For example some scores use parameters such as blood pressure measured on admission and troponin (GRACE) whilst others do not specify.

There are several other risk models which have been developed with varying degrees of validation across a variety of patient cohorts, *e.g.*, All ACS or all PCI. Others have been developed in an era which do not reflect contemporary practice, *e.g.*, The Primary Angioplasty in

Myocardial Infarction score (PAMI)^[35]. These will not be reviewed in detail in this manuscript as they are of limited clinical applicability, and have often excluded the highest risk patients such as the National Cardiovascular Data Registry (NCDR) PCI risk score^[36].

BLEEDING RISK SCORES

Bleeding is an important outcome of ACS. The majority of patients with ACS will receive anti-coagulants and dual anti-platelet therapy and some patients will receive fibrinolysis or PCI that increase bleeding risk. There are limited data on bleeding risk scores in the setting of PPCI. The CRUSADE bleeding risk score (CBRS, Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA Guidelines) has been utilised and validated in a NSTEMI population but not in the STEMI cohort^[37]. A prospective study from Spain has suggested that the bleeding risk in patients with PPCI in their cohort was less than that of the NSTEMI group. The lower rate of bleeding observed in this group may be due to the cohort having a lower baseline risk (younger, predominantly male) there was also a lower incidence of cardiovascular disease. A radial approach for PCI was associated with a decreased risk of major bleeding although the exact cause for this is unclear. This study lacked data on contemporary practice as patients on newer antiplatelet agents such

as Ticagrelor were excluded^[38].

PPCI outcome-survival

In contemporary practice, survival rates following PPCI are high and approach 95% to 97% at 3 years^[13,19,40]. However, within this group there is a wide range of individuals with varying levels of underlying risk. The elderly have worse absolute outcomes compared to their younger counterparts. In the APEX-AMI study the 90-d mortality was 13.1% in the elderly (> 75 years) and 2.3% in the < 65 years cohort. In this study age was the strongest predictor of mortality (hazard ratio 2.07 per 10 year increase (95%CI: 1.84-2.33)^[41,42].

Mitigating against this absolute higher mortality is the fact that the elderly have a higher baseline risk and their relative risk is reduced by PPCI more effectively than by fibrinolysis. In the elderly STEMI population this has been demonstrated in the TACTICS-TIMI 18 trial in which there was a greater absolute risk benefit in favour of revascularisation^[43]. Registry data support this finding, in the Australian ACACIA registry decreased referral rate and rate of revascularisation was noted in the elderly population. The exact reason for this is not clear; however it may be due to a perceived increase in risk by referring physicians or judgements based on frailty. In the same registry there was increased absolute benefit to early revascularisation in the elderly compared to the young following adjustment for baseline risk^[44].

A final limitation of studies that report all-cause mortality is a failure to consider that longer-term survival may be affected by non-cardiac pathology. These factors may influence outcome beyond the index STEMI event. The elderly population are exposed to increased mortality attributable to non-cardiovascular factors than compared with their younger counterparts whether they have recovered from STEMI or not^[45].

PPCI outcome-absolute risk reduction

The impact of any treatment is dependent on the baseline risk. The relative risk reduction of treatment in a low risk group is small and the number needed to treat (NNT) is high, this was illustrated in the In the PCAT-2 collaboration (Primary Coronary Angioplasty Trialist versus Thrombolysis) where the NNT with PPCI for a lowest quartile was 516 compared with 17 in the highest risk quartile. A patient with a risk score of 5 would decrease their absolute risk by 10% whereas the patient with a risk score of 1 would decrease their absolute risk by less than 1%^[46]. Yet the potential benefit of PPCI must also be considered in context of the risk of harm. In a young age group the risk of bleeding from fibrinolysis is low whereas the elderly have a higher incidence of intracranial bleeding^[47].

The challenge of optimally treating high-risk patients is exacerbated by the increased prevalence of an atypical presentation. A failure or delay to make a diagnosis prevents risk evaluation and reduces the benefit of treatment, up to 90% of patients under the age of 65 present with chest pain *vs* 57% over 85 years^[40]. Elderly patients

are more likely to present with atypical features such as left bundle branch block (34%), acute heart failure without significant chest pain (45%) all of which may delay diagnosis. In the real world delays in diagnosis and access to treatment are common and contribute to harm. Some authors advocate tailoring trials and treatment specifically to include the elderly high risk cases^[45,47,48].

PPCI-important secondary outcomes

Post infarct complications other than mortality are important factors in determining overall efficacy. Ghara-cholou *et al*^[27] showed that compared to their younger counterparts the elderly have a higher baseline risk and a higher rate of post infarct/PPCI complications, in particular stroke (1.5% *vs* 0.4%), CCF (11.5% *vs* 2.7%) and shock (6.9% *vs* 2.1%). After correction for baseline characteristics age was a predictor of death (HR = 2.07; 95%CI: 1.84-2.33, *P* < 0.001)^[41]. For high risk elderly patients there are no randomised trials to guide optimal management. Inferences about management have been drawn from analysis of sub-groups from PPCI trials^[51].

Hospital length of stay is less following PPCI than with fibrinolysis (3 d *vs* 5 d)^[50]. But there is relatively little data on quality of life in STEMI patients beyond 1 year and no data on the relative quality of life between high risk patients (often the elderly) and lower risk patients. Recent data from the GRACE registry suggests favourable 5 year survival but there are no long term data for quality of life following PPCI in either the younger or elderly group^[49].

Recently the United Kingdom National Health Service has begun to focus attention on this by introducing measures of patient report experiences and outcomes. There is some evidence (outwith PPCI) that while it may provide more information it does not necessarily alter clinicians management strategies^[52]. Data from the FREEDOM study (Future Revascularization Evaluation in Patients With Diabetes Mellitus: Optimal Management of Multivessel Disease) has suggested quality of life benefits for PCI at 2 years however these were in chronic stable angina patients^[53].

THE IDEAL PPCI RISK SCORE

A discriminatory risk score is required when the effectiveness of treatment depends on baseline risk. An optimal risk score for PPCI would predict which patient would benefit maximally from an intervention and predict who would come to harm and what weighting should be ascribed to that. The risk of death and morbidity in the context of an anterior STEMI is high and reperfusion treatment with thrombolysis or PPCI outweighs the risk of bleeding in most patients. Conversely the risk of harm in a late presenting or limited inferior STEMI may outweigh the perceived benefits of reperfusion treatment and conservative treatment could be advocated.

Currently there is no risk scoring system within the context of STEMI and physicians are encouraged to rapidly activate a treatment pathway with little or no as-

assessment of perceived risks and benefits. The reasons for this practice have been discussed and are summarised by a lack of guideline recommendation, impractical or non-discriminatory scoring systems and a perception that all STEMI patients are high risk. A further limitation is that the clinical trials on which evidence is based are highly selective samples. Typically these trials recruit less than 10% of patients screened and often the very highest risk patients are excluded. This has the effect of excluding 'real world' patients from evaluation of interventions. Any scoring system derived from a clinical trial by default is not applicable to a real world population. A lack of applicability of trial data to the real world is often cited as a reason to not offer therapies. Trials performed in highly selected patients that show efficacy of treatment may drive the widespread delivery of this treatment to an "all-comers" population. This may be effective but may not be cost effective. The same treatment (PPCI) may be offered for example, to a 40-year-old male presenting within 60 min of onset of STEMI. Currently PPCI would be offered, with a number needed to treat of > 500 to save one life. Thrombolysis delivered immediately may be as effective with little chance of harm. Conversely a late presenting elderly female who has a much higher risk of death, lower likelihood of reperfusion with fibrinolysis, higher rate of significant bleeding and therefore is much more likely to benefit from mechanical reperfusion, number needed to treat = $17^{[46]}$.

Opportunities and missed opportunities of care

Is the current philosophy of STEMI treatment correct? PPCI has been calculated to cost the NHS in England £5176 per patient during office hours versus fibrinolysis at £3509^[54,55]. This represents a significant burden of healthcare resource devoted to a treatment that in some patients is probably life saving in many others not. There is little licence or encouragement for physicians to discriminate between these very different patient groups and the mandate is to treat rapidly. However, there is no doubt that this approach has been effective and real world survival following STEMI treated by PPCI is remarkably high.

Can refinement with risk adjustment improve the pathways further? Clearly the determination of absolute risk and absolute benefit in high-risk populations is difficult, as is proving that the elderly benefit in the long term from intervention and aggressive secondary prevention. One of the challenges confronting front line clinicians is lack of clear prognostic data that takes into account the patient as a whole and not simply their acute STEMI presentation. The idea of assessing potential harm as well as possible/likely benefit has recently been given increased attention.

In the United States the wide disparity of care has in recent years been highlighted. There is considerable variation in practice both in geographical terms and in differing financial arrangements. Since the introduction of The Affordable Care Act (ACA, Obamacare) a substantial amount (United States \$1.1 billion) of the United States health budget has been appropriated to funding towards

Comparative Effectiveness Research. The intention being to improve quality, streamline care and demonstrate not only medical efficacy but medical effectiveness. This alteration in the funding landscape has profound implications for physician choice and may influence clinical decision making. Some authors have suggested that it may lead to creeping government control of medical practice by influencing reimbursement^[56]. This is analogous to the system in the United Kingdom where NICE delivers guidelines based both on treatment efficacy and overall clinical effectiveness. While this system has its merits in trying to alleviate some of the problems associated with the so called "postcode lottery" NICE is not empowered to make funding allocations although patients have a right to NHS approved treatments NICE recognises that further research is recommended into optimal reperfusion strategies for those presenting early.

In contrast to the front loading of healthcare provision at the time of presentation with STEMI there remains a significant failure in prescribing simple evidence based treatments following the initial treatment. Provision of secondary prevention pharmacotherapy has been described using a missed opportunities for care model. A study using a large United Kingdom national database (MINAP) which demonstrated that outcome (death) was related to not prescribing clinically indicated and evidence based treatments, *e.g.*, statins^[57]. In another study of elderly patients the authors found that following PCI healthcare inequalities expressed as missed opportunities for care in the short term (30 d) correlated with mortality^[58].

Efficacy of treatment

The MINAP based study result above illustrates the importance of proof of benefit and not simply reduction of risk^[57,58]. The efficacy of secondary preventative medication in a population has been established. What is less clear is the prognostic benefit in high risk individuals. We have already seen above that missed opportunities equate to outcome.

As pressure on healthcare budgets have come under increased scrutiny, research methodology, *e.g.*, high cost of Randomised Control Trials (RCTs) have come under review. This has reinvigorated interest in research methods that provide prognostic information. Comparative effectiveness research has been suggested as a possible route towards improving outcomes and reducing costs whilst providing policy makers and clinicians with clinically useful and evidence based tools to achieve optimal care. An example of this would be the use of electronic medical records to generate evidence from different areas and compare outcomes based on geographical locations^[59]. Alternatively a design similar to the recent STREAM study when ethical approval was granted for a centre to conduct a trial of early fibrinolysis *vs* PPCI for early presenters^[18].

CONCLUSION

Clinicians are generally poor at judging risk and predicting the absolute benefit and harm of their interventions.

The evidence in NSTEMI care has clearly shown the importance of calculating these metrics. This has led to a plethora of risk scores and recommendation to use these in international guidelines.

Provision of STEMI care in the United Kingdom is currently algorithmic and not risk adjusted yet we have seen that the same treatment pathway (PPCI) may deliver treatment that is very beneficial in some but not in others. One reason to risk stratify is to target healthcare resource; many patients should continue to be treated by emergency PCI, others may be treated with immediate fibrinolysis and others without reperfusion treatment at all.

The STREAM and GRACIA-2 data have suggested that some patients can be treated as effectively and certainly more cost effectively with rapid thrombolysis avoiding emergency angiography^[14,17]. These data come from trials and consequently have all the limitations of selection and applicability but have generated an important hypothesis. If discriminatory STEMI risk scores were available, applicable to real world patients and widely used could the current algorithm of emergency angiography be adapted to include fibrinolysis? If this change were incorporated would the outcomes be non-inferior or the cost benefit calculation superior. There are huge challenges to proving this hypothesis. Some clinicians will feel that such a change is retrograde step and there is a risk of generating a complicated pathway that may harm the very patients it is intending to improve outcomes for. The trials involved to mark such a paradigm shift in the current guidelines may be costly, difficult to recruit to and may not provide a definitive answer. Thus the question “Would this change be non-inferior to PPCI overall and would it be cost beneficial” may be difficult to answer. The first step is to generate a practical discriminatory risk score that is based on real world data in a STEMI population. Ideally the score should account for potential harm associated with PCI or thrombolysis, should generate baseline risk and calculate treatment effects. Such a risk score does not yet exist although registry data are available on which these could be derived. A validated score has ability to predict the impact of healthcare on treatment and evaluate cost-benefit.

If substantial health care resource is being driven towards treatments that are only minimally effective in some patients then refinement of the STEMI pathway by risk adjustment should be formally evaluated. There are merits to keeping treatment pathways simple and providing algorithmic care if this is globally effective. However stratifying patients by risk and calculating treatment effects with thrombolysis or PCI may be as effective. Such a pathway could be delivered with reduced overall cost and no less efficacy^[60].

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Late intervention in an asymptomatic pediatric patient with anomalous left coronary artery

John C Lam, Michael Giuffre, Kimberley A Myers

John C Lam, Michael Giuffre, Kimberley A Myers, Department of Pediatrics, University of Calgary, Alberta Children's Hospital, Calgary, Alberta T3B 6A8, Canada

Author contributions: Giuffre M and Myers KA attended the patient; all authors prepared the manuscript and figures and read and approved the final manuscript.

Correspondence to: Michael Giuffre, MD, MBA, FRCPC, FACC, Clinical Professor, Department of Pediatric Cardiology, University of Calgary, Alberta Children's Hospital, 2888 Shaganappi Trail NW, Calgary, Alberta T3B 6A8, Canada. michael.giuffre@albertahealthservices.ca

Telephone: +1-403-2552737 Fax: +1-403-2556709

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the pulmonary artery and the associated challenge with determining the need for surgical revascularization in the absence of symptomatology and definitive literature. Late surgical intervention undertaken in this patient may reverse ongoing myocardial dysfunction and prevent permanent left ventricular damage.

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Abstract

Anomalous left coronary artery from the pulmonary artery (ALCAPA) is most commonly diagnosed within the first year of life with congestive heart failure symptomatology reflecting left ventricle (LV) dysfunction. The late diagnosis of ALCAPA is presented in a 5-year-old without significant LV dysfunction, mild LV dilatation and only mild mitral regurgitation that did not change significantly after surgery. The timing of surgical intervention in the late diagnosis of ALCAPA remains unclear despite risks of significant ongoing myocardial injury secondary to coronary artery hypoperfusion and progressive mitral valve dysfunction. Intervention in this case allows for revascularization which may reverse ventricular and valvular dysfunction.

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Key words: Congenital heart disease; Congenital heart surgery; Coronary artery imaging; Coronary artery surgery; Reperfusion

Core tip: This case report presents the rare case of a late diagnosis of anomalous left coronary artery from

INTRODUCTION

This case report describes a late diagnosis of anomalous left coronary artery from the pulmonary artery (ALCAPA) in an asymptomatic patient with only mild left ventricular dilatation. Because of a lack of literature to guide management in our patient, the benefits of surgical revascularization were weighed against risks of surgery on a hemodynamically stable patient with relatively preserved ventricular function. Ultimately, the patient underwent surgery in hopes of preventing further suboptimal coronary perfusion.

CASE REPORT

The patient was initially seen with an asymptomatic 2/6 apical systolic murmur at age two. The electrocardiogram was normal and the initial transthoracic echocardiogram demonstrated good ventricular function, no atrial or ventricular septal defect, and mild mitral regurgitation (MR). There was abnormal flow in the region of the left coronary artery (LCA) suspicious for a LCA fistula.

To further visualize the LCA, a transesophageal echocardiogram performed at age two under general anesthesia confirmed normal left and right ventricular function, a

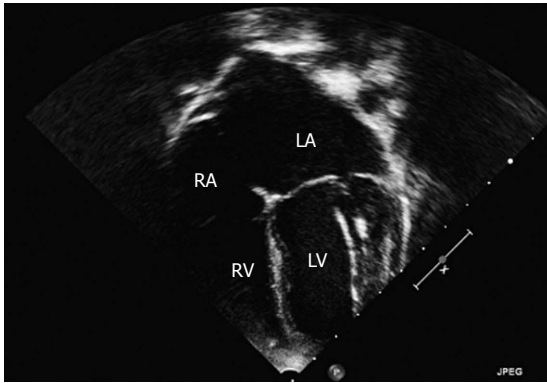


Figure 1 Four-chamber echocardiographic images illustrating echogenic left papillary muscle and chordae apparatus. LV: Left ventricle; LA: Left atrium; RA: Right atrium; RV: Right ventricle.

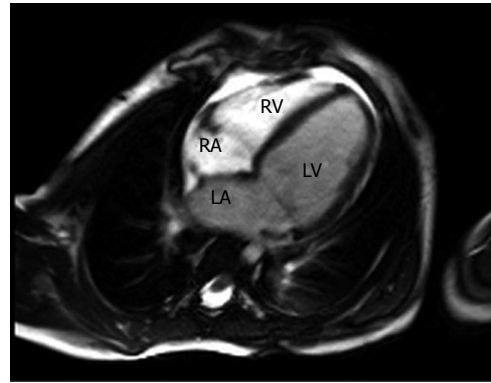


Figure 2 Four-chamber cardiac magnetic resonance cine imaging demonstrating a dilated left atrium and left ventricle. LV: Left ventricle; LA: Left atrium; RA: Right atrium; RV: Right ventricle.

small LCA fistula and mild MR with normal ventricular chamber dimensions. Repeated echocardiograms showed evidence of mild dilatation of the left atrium (LA) and left ventricle (LV) with mild MR. Increased echogenicity of the LV myocardium was noted but there was no evidence of endocardial fibroelastosis on these pre-operative echocardiograms (Figure 1). At age 5 further investigation with cardiac magnetic resonance imaging (MRI) saw findings consistent with the previously noted dilated LA and LV, accompanied by normal LV systolic function and mild MR (Figure 2). The LCA system was suboptimally visualized by cardiac MRI but raised the question of an anomalous LCA. Cardiac computed tomography was then performed with angiography demonstrating ALCAPA. Numerous collateral vessels from the right coronary artery to the left coronary system, along with a dilated LA and LV were visualized (Figure 3).

Surgical repair was undertaken with a direct aortic reimplantation performed under cardiopulmonary bypass after arresting the heart with cold blood cardioplegia. The left coronary button was excised and anastomosed with a flap of ascending aorta. The pulmonary root was reconstructed with bovine pericardium before anastomosis to the main pulmonary artery (PA). The patient was weaned from cardiopulmonary bypass uneventfully after a total bypass time of 78 min.

Post-operatively, the patient was hemodynamically stable spending one night in the pediatric intensive care unit. Brief episodes of hypotension were managed routinely with inotropes and intravenous fluid. She was discharged ten days after surgery, weaned off oxygen and prescribed enalapril and aldactazide. Her post-operative course was complicated by mild postpericardiotomy syndrome that responded to acetylsalicylic acid therapy.

The immediate post-operative echocardiograms demonstrated mild MR along with mild dilatation of the LA and LV, and a small pericardial effusion. The patient's LV function remained preserved with the shortening fraction improved from 35% to 40%. At her two-month post-operative assessment, she was asymptomatic, remaining on low dose enalapril. Her echocardiogram showed mild LA dilatation, mild LV dilatation, and two separate jets of

mild MR. Ventricular function was relatively unchanged with a shortening fraction of 38% and ejection fraction of 69%.

Structural assessment of the LV demonstrated a quantitative reduction in LV dilatation following the procedure with the left ventricle internal diameter-diastole reduced from 4.4 cm pre-operatively to 3.9 cm one month after surgery. The degree of MR pre and post-operatively had not changed, with repeat assessment scheduled at six months post-operatively.

DISCUSSION

ALCAPA is rare congenital anomaly in which the LCA receives deoxygenated blood via the PA. It does not present as a hemodynamic problem in utero as high pulmonary vascular resistance allows for antegrade blood flow from the PA into the LCA. As the pulmonary vascular resistance falls, retrograde flow into the PA develops producing coronary steal or hypoperfusion.

ALCAPA has an incidence of 1/300000 live births^[1] and its symptoms of failure to thrive, dyspnea, diaphoresis and findings of left sided heart failure usually present within the first two months of life^[2]. Such early diagnoses of ALCAPA usually require prompt surgical revascularization. Without surgery, 90% of infants with ALCAPA will often die in the first year of life due to myocardial ischemia secondary to coronary vessel hypoxia^[3].

In order to survive, collateral vessels are necessary to supply the LV myocardium. However, the ALCAPA continues to place stress on the LV, resulting in steadily deteriorating systolic function and LV scarring. Our patient's MR secondary to subclinical decline of her LV function indicated that the structure of her LV was indeed a concern despite her collateral vasculature. The mildly increased echogenicity of the LV endocardium raised the concern of endocardial fibroelastosis eventually developing with resultant myocardial scarring. This led to the decision for surgery rather than careful cardiac follow-up.

Surgical revascularization is the treatment of choice for ALCAPA. The surgery is most often scheduled immediately after diagnosis of ALCAPA in order to prevent

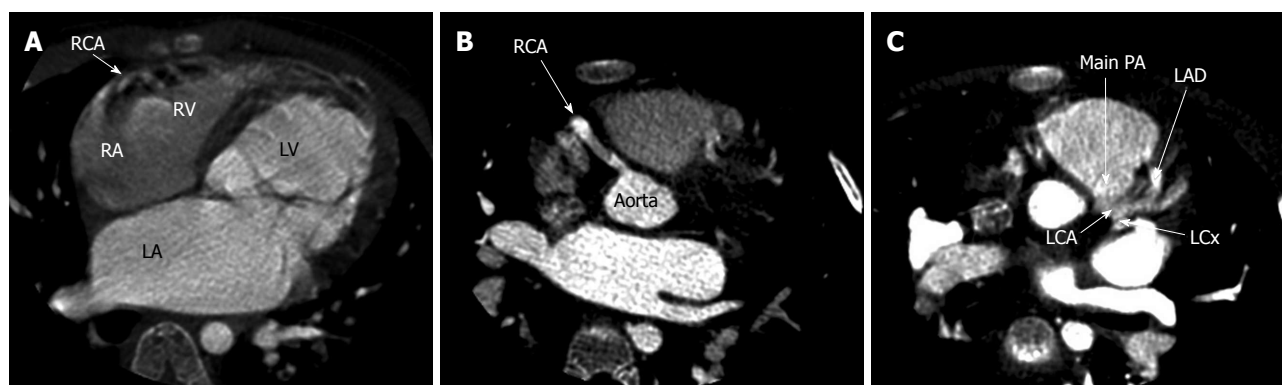


Figure 3 Computed tomographic angiography. A: Computed tomographic angiography demonstrating extensive collateral vessels from the right coronary artery as well as a dilated left ventricle; B: Computed tomographic angiography showing dilated right coronary artery; C: Computed tomographic angiography illustrating the left anterior descending artery communicating with the main pulmonary artery. LV: Left ventricle; LA: Left atrium; RA: Right atrium; RV: Right ventricle; RCA: Right coronary artery; LAD: Left anterior descending artery; LCA: Left coronary artery; LCx: Left circumflex artery; PA: Pulmonary artery.

further ischemia and necrosis of cardiac muscle. Most surgeries involve either a Takeuchi operation or direct re-implantation of the LCA^[3].

The role of revascularization in an asymptomatic patient with functional collateral coronary vessels and years of subclinical myocardial ischemia remains somewhat contentious. Literature in the adult population suggests that myocardium supplied by longstanding ischemic vasculature may be permanently scarred and unable to regain function despite intervention^[4]. This did not appear to be the case with our 5-year-old patient. Surgical intervention places patients at risk of arrhythmias, acute cardiac failure and long-term complications such as intrapulmonary tunnel baffle leaks and suprapulmonary stenosis^[3]; however, these did not occur in the case presented, but may develop over more time.

Recovery of LV function is well reported in pediatric patients following revascularization. However, our patient maintained normal ventricular function. Early diagnosis and intervention is correlated with a decreased need of mitral valve repair or replacement in the future^[5]. Asymptomatic patients with unrepaired ALCAPA have chronic ischemia despite the production of collateral coronary vessels. The LV continues to undergo adverse remodeling with deterioration in systolic and diastolic function over time. Several studies have demonstrated successful recovery of LV function in patients following surgical intervention for ALCAPA^[6,7]. However, patients with poor LV function pre-operatively have higher mortality rates and increased need for mitral valve intervention after ALCAPA surgery.

In the setting of decreased LV function, there is hypothesis regarding the possible existence of hibernating myocardium in ALCAPA. The hypothesis suggests myocytes supplied by the ALCAPA undergo an adaptive change and become hypokinetic rather than necrotic despite being hypoperfused^[8]. The function and viability of the cardiomyocytes is preserved because of the successful formation and utilization of a compensatory but inadequate collateral supply. Shivalkar *et al*^[2] demonstrated that although chronically hypoperfused, histology of myocar-

dium from some patients with ALPCA is comprised of viable myocytes with minor morphological changes likely from cellular adaptation to longstanding ischemia.

This case illustrates the considerations in revascularizing an asymptomatic ALCAPA patient with longstanding hypoperfused myocardium but preserved ventricular function in the absence of definitive literature. Surgical intervention was recommended to prevent the potential of further LV dysfunction and mitral valve dysfunction manifesting from further suboptimal myocardial perfusion. The late diagnosis of ALCAPA in an asymptomatic 5-year-old with preserved LV function, mild LV dilatation and mild MR that did not change after surgery further illustrates the clinical dilemma of the timing of surgical intervention.

In conclusion, this case describes the late presentation of a child with subclinical myocardial ischemia secondary to ALCAPA. The patient compensated well hemodynamically for five years with only mild LV dilatation and mild MR. The need for surgical intervention considered the risks of cardiopulmonary bypass on scarred LV myocardium versus the benefits of repair and potential reversal of ongoing myocardial dysfunction and LV myocardial damage, but the timing of such surgery remains somewhat controversial.

COMMENTS

Case characteristics

A late diagnosis and operative revascularization procedure was performed in a 5-year-old female.

Clinical diagnosis

The patient presented with an asymptomatic apical systolic murmur.

Differential diagnosis

Left coronary artery fistula, anomalous left coronary artery arising from the pulmonary artery

Imaging diagnosis

Cardiac computed tomography with angiography demonstrated an anomalous left coronary artery from the pulmonary artery.

Treatment

Surgical revascularization with a direct aortic reimplantation was performed under cardiopulmonary bypass.

Related reports

The role of revascularization in an older asymptomatic patient with anomalous left coronary artery from the pulmonary artery (ALCAPA) is unclear as there lacks strong clinical literature to guide timing of treatment.

Experiences and lessons

Late surgical intervention ALCAPA may reverse ongoing myocardial dysfunction and prevent permanent left ventricular damage, even in hemodynamically stable patients.

Peer review

The authors present a rare case report of late diagnosed ALCAPA. Surgical intervention was undertaken and left ventricular function was relatively preserved. The authors emphasized the importance of the timing of surgical intervention from the viewpoint of risks and benefits. The manuscript is clearly written and well organized.

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World Journal of *Cardiology*

World J Cardiol 2014 September 26; 6(9): 878-1048



**TOPIC HIGHLIGHT**

- 878** African Americans, hypertension and the renin angiotensin system
Williams SF, Nicholas SB, Vaziri ND, Norris KC
- 890** Metabolic syndrome in hypertensive patients: An unholy alliance
Mulè G, Calcaterra I, Nardi E, Cerasola G, Cottone S
- 908** Management of erectile dysfunction in hypertension: Tips and tricks
Viigimaa M, Vlachopoulos C, Lazaridis A, Doulmas M
- 916** Multimodality imaging in apical hypertrophic cardiomyopathy
Parisi R, Mirabella F, Secco GG, Fattori R
- 924** Thrombus aspiration in acute myocardial infarction: Rationale and indication
Sardella G, Stio RE
- 929** Drug-eluting stents and acute myocardial infarction: A lethal combination or friends?
Otsuki S, Sabaté M
- 939** miRNome in myocardial infarction: Future directions and perspective
Boštjančič E, Glavač D

REVIEW

- 959** Ventricular tachycardia mapping and ablation in arrhythmogenic right ventricular cardiomyopathy/dysplasia: Lessons Learned
Tschabrunn CM, Marchlinski FE
- 968** Angiotensin II-related hypertension and eye diseases
Marin Garcia PJ, Marin-Castaño ME
- 985** Is ABO blood group truly a risk factor for thrombosis and adverse outcomes?
Zhou S, Welsby I
- 993** Cardiac manifestations in systemic sclerosis
Lambova S
- 1006** Serum creatinine role in predicting outcome after cardiac surgery beyond acute kidney injury
Najafi M

- 1022** Newer methods of cardiac output monitoring

Mehta Y, Arora D

**OBSERVATIONAL
STUDY**

- 1030** Does manual thrombus aspiration help optimize stent implantation in ST-segment elevation myocardial infarction?

Fernández-Rodríguez D, Alvarez-Contreras L, Martín-Yuste V, Brugaletta S, Ferreira I, De Antonio M, Cardona M, Martí V, García-Picart J, Sabaté M

CASE REPORT

- 1038** Calcific left atrium: A rare consequence of endocarditis

Dattilo G, Anfuso C, Casale M, Giugno V, Camarda L, Laganà N, Di Bella G

- 1041** Systemic venous atrium stimulation in transvenous pacing after mustard procedure

Puntrello C, Lucà F, Rubino G, Rao CM, Gelsomino S

- 1045** Acute myocarditis triggering coronary spasm and mimicking acute myocardial infarction

Kumar A, Bagur R, Béliveau P, Potvin JM, Levesque P, Fillion N, Tremblay B, Larose É, Gaudreault V

Contents

World Journal of Cardiology
Volume 6 Number 9 September 26, 2014

APPENDIX I-V Instructions to authors

ABOUT COVER Editorial Board Member of *World Journal of Cardiology*, Tzong-Shyuan Lee, DVM, PhD, Professor, Department of Physiology, National Yang-Ming University, Taipei 11221, Taiwan

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WJC 6th Anniversary Special Issues (1): Hypertension

African Americans, hypertension and the renin angiotensin system

Sandra F Williams, Susanne B Nicholas, Nosratola D Vaziri, Keith C Norris

Sandra F Williams, Department of Endocrinology, Cleveland Clinic, Weston, FL 33331, United States

Sandra F Williams, Department of Clinical Biomedical Science, Charles E Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL 33431, United States

Susanne B Nicholas, Division of Nephrology and Division of Endocrinology, Diabetes and Hypertension, Department of Medicine David Geffen School of Medicine, University of California, Los Angeles, CA 94305, United States

Nosratola D Vaziri, Division of Nephrology and Hypertension, Departments of Medicine, Physiology and Biophysics, University of California, Los Angeles, CA 94305, United States

Keith C Norris, Division of General Internal Medicine and Health Services Research, Los Angeles, CA 90024, United States

Keith C Norris, Department of Medicine David Geffen School of Medicine, University of California, Los Angeles, CA 90024, United States

Author contributions: All authors contributed to this work.

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Correspondence to: Keith C Norris, MD, FASN, Professor of Medicine, Division of General Internal Medicine and Health Services Research, 911 Broxton Ave, Room 103, Los Angeles, CA 90024, United States. kcnorris@mednet.ucla.edu

Telephone: +1-310-7946973 Fax: +1-310-7940732

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Abstract

African Americans have exceptionally high rates of hypertension and hypertension related complications. It is commonly reported that the blood pressure lowering efficacy of renin angiotensin system (RAS) inhibitors is attenuated in African Americans due to a greater likelihood of having a low renin profile. Therefore these agents are often not recommended as initial therapy in African Americans with hypertension. However, the high prevalence of comorbid conditions, such as diabe-

tes, cardiovascular and chronic kidney disease makes treatment with RAS inhibitors more compelling. Despite lower circulating renin levels and a less significant fall in blood pressure in response to RAS inhibitors in African Americans, numerous clinical trials support the efficacy of RAS inhibitors to improve clinical outcomes in this population, especially in those with hypertension and risk factors for cardiovascular and related diseases. Here, we discuss the rationale of RAS blockade as part of a comprehensive approach to attenuate the high rates of premature morbidity and mortality associated with hypertension among African Americans.

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Key words: African American; Blood pressure; Ethnicity; Hypertension; Renin; Angiotensin

Core tip: African Americans have exceptionally high rates of hypertension and hypertension related complications. Due to a greater likelihood of having a low plasma renin levels, inhibitors of the renin angiotensin system (RAS) are often not recommended as initial antihypertensive therapy. However, animal models suggest hypertension characterized by low circulating renin levels have a paradoxical increase in tissue RAS activity. Thus treatment with RAS inhibitors may be critical to preventing end organ damage. We describe the rationale of RAS blockade as part of a comprehensive approach to attenuate the high rates of premature morbidity and mortality associated with hypertension among African Americans.

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INTRODUCTION

Hypertension is characterized by a persistent and frequently progressive elevation in blood pressure^[1]. The level of systolic blood pressure (SBP) and/or diastolic blood pressure (DBP), which connotes a diagnosis of hypertension, may vary depending on the presence or absence of coexisting comorbidities^[1,2]. Hypertension is commonly defined as physician diagnosed SBP ≥ 140 mmHg and DBP ≥ 90 mmHg; and pre-hypertension is defined as SBP ≥ 120 mmHg and < 140 mmHg or DBP ≥ 80 mmHg and < 90 mmHg^[1]. However, a recent report from the panel members appointed to the Eighth Joint National Committee recommended a SBP goal of < 150 mmHg and DBP goal < 90 mmHg in persons ≥ 60 years of age without diabetes mellitus (DM) or chronic kidney disease (CKD)^[3]. The committee also recommended that in the African American hypertensive population, including those with diabetes, initial therapy should begin with a calcium channel blocker or thiazide-type diuretic, but acknowledged that there was modest evidence for renin-angiotensin system (RAS) inhibition as initial or add-on antihypertensive therapy in African Americans with CKD^[3]. However, a minority of the committee members did not support a higher SBP goal at age ≥ 60 years or the choices for initial antihypertensive therapy in African Americans. They were particularly concerned that the newly recommended higher blood pressure goal may adversely affect patients aged ≥ 60 years with cardiovascular disease (CVD) risk factors other than DM or CKD^[4]. In addition, they interpreted the evidence supporting an increase in the SBP target from < 140 mmHg to < 150 mmHg in persons 60 years of age or older as insufficient and inconsistent with the evidence supporting the panel's recommendations for an SBP target of < 140 mmHg in younger persons^[4]. There is also the risk that as the new guidelines are disseminated that the "take home" messages may miss the nuances such as the new recommended higher goal in persons ≥ 60 years of age excluding persons with DM or CKD, and that with a goal of < 150 mmHg many patients may spend much of their time with their blood pressure (BP) above that level.

In the United States African Americans develop hypertension at an earlier age than whites, have much higher average blood pressure readings, a greater likelihood of refractory hypertension, and greater rates of premature hypertensive complications such as CKD, stroke and heart disease^[5-7]. Importantly, African Americans suffer from a three-fold higher death rate from hypertension with cardiovascular complications accounting for the majority of deaths^[5]. Data from the National Health and Nutrition Examination Survey (NHANES) indicate/show that although there has been a trend toward improving BP control among African Americans, the overall control of BP remains suboptimal at a national level. During the period from 1999-2004, blood pressure was adequately controlled in only 35% of whites, 29% of African Americans, and 27% of Hispanics^[8]. By 2007-2008

blood pressure control rates had increased to 50% and for the first time were now similar across race/ethnicity^[9]. While this is a marked improvement, it still represents half of Americans with hypertension having poor blood pressure control. However other data underscore the lingering concern for BP control as a significant problem among African Americans. In the Multi-Ethnic Study of Atherosclerosis (MESA) the percentage of treated but uncontrolled hypertension was significantly higher in African Americans (35%), Chinese (33%), and Hispanics (32%) than in whites (24%)^[10]. After adjustment for clinical and socioeconomic factors the relative higher rates of uncontrolled hypertension for Chinese and Hispanic participants largely disappeared, but persisted in the African American population, suggesting an independent effect to account for these observed differences such as other biologic and/or non-biologic factors not assessed in the MESA study^[10].

BIOLOGIC FACTORS INFLUENCING HYPERTENSION IN AFRICAN AMERICANS

The reasons for the exceptionally high rates of hypertension and associated end organ damage among African Americans, are not entirely clear but likely include socioeconomic status, lifestyle choices, clinical factors (*e.g.*, increased risks of diabetes and hypertension), environmental, and biologic/genetic factors that may contribute broadly to racial/ethnic differences in not only outcomes but in response to therapeutic intervention^[11-17]. Our understanding of the complex factors that predispose African Americans to hypertension and hypertension-related complications is evolving. While RAS inhibition has emerged as an important antihypertensive therapy, the blood pressure lowering efficacy of RAS inhibitors is attenuated in African Americans^[18], likely due to an increased prevalence of having a low renin profile. Therefore these agents may be less likely to be recommended as initial therapy in African Americans with hypertension^[3].

One major biologic factor that is highly relevant to RAS inhibition is the particularly high prevalence of salt sensitivity in African Americans, which is also associated with a low circulating plasma renin profile^[19-26]. Salt sensitivity is defined as an increase in blood pressure in response to sodium or salt intake, and is commonly associated with a low circulating renin profile^[24,27,28]. Wilson *et al.*^[29] postulated that the slave trade from Africa to the Americas led to extreme volume depletion and cardiovascular collapse during the journey due to diarrheal diseases and limited access to water favoring the survival of persons who were avid sodium retainers and accelerating gene selection for sodium retention. Consistent with this premise, Maseko *et al.*^[30] found no relationship between blood pressure and 24-h urinary sodium and potassium excretion rates in nearly 300 city-dwelling black South Africans, suggesting African slave descendants have a

higher rate of salt sensitivity than native black Africans. However many of the historical claims which form the basis of the slavery hypothesis for hypertension have been challenged by other authors^[29,31]. In question are the key tenets of the theory which implicate salt deficiency in the areas of Africa from which slaves originated, the trauma of the slave trade, and conditions in the Americas as triggers for unnatural genetic selection for renal sodium-retainers^[29,32]. According to this theory, these factors collectively evolved into the eventual present-day disproportionately higher blood pressure in African Americans compared with their counterpart whites or African blacks in modern sodium-rich societies^[29,31,32]. In the absence of access to records of past salt availability in Africa, slave trade disease states, and dietary salt content in the Americas between the 16th and 19th century, it will be impossible to ever fully confirm or fully refute this hypothesis^[33]. However, others have refuted the slave trade hypothesis as contributing to excess rates of hypertension in blacks of African descent^[31].

Some specific features of salt-sensitive hypertension have been characterized. Evidence suggests that the sympathetic nervous system may play a role in the modulation of salt sensitivity. In the presence of salt loading, the typical response of the sympathetic nervous system is to decrease norepinephrine, a known sodium retainer, and to increase dopamine which promotes sodium excretion^[34,35]. However in salt sensitive hypertensive individuals, particularly African Americans, there appears to be a dysfunctional response of the sympathetic nervous system in the presence of excess salt. In these patients, salt loading is associated with decreased urinary dopamine levels and the absence of any significant decreases in norepinephrine^[35,36]. Another factor which is implicated as accounting for differences in salt sensitivity is kallikrein which has been demonstrated to be excreted in lower levels in salt sensitive hypertensive persons, particularly African Americans^[37,38]. Whether the fact that African Americans consume less potassium, a known kallikrein releaser, is contributory, remains unclear^[24,37]. Evaluation of the relationship between potassium intake, urinary kallikrein levels and salt sensitivity is warranted using large scale clinical trials.

Among the non-biologic factors which could possibly explain the inequity in the occurrence of hypertension among the races, obesity remains a tempting option given its similar trend of increased prevalence in African Americans. Excess adiposity, a reflection of lifestyle habits, has a reported 51% greater prevalence in this population. In addition, there has been an association reported between obesity, insulin resistance and other adipokine-mediated pathways with the occurrence of salt-sensitivity^[39]. However, NHANES data from the 1988-1994 time period show no significant difference in obesity between white men and black men (20.3% and 21.1% respectively) while there was a 46% greater prevalence of hypertension in black men over white men during that same period^[39]. Also, Okosun *et al*^[40] have shown that the risk of African

American race for high blood pressure remains after adjusting for abdominal adiposity in NHANES III data. Therefore the evidence does not support obesity as the sole contributor to the disparity in prevalence of hypertension among blacks although it is not excluded as a contributory factor.

RENIN-ANGIOTENSIN SYSTEM IN SALT SENSITIVE HYPERTENSION

The disproportionate burden of both hypertension and its sequelae in African Americans underscores the significance of optimizing approaches to blood pressure control in order to prevent and/or attenuate the high rate of hypertensive complications. A comprehensive understanding of the role of RAS blockade as part of a strategy for blood pressure control and attenuation of end organ disease is critical to selecting therapeutic agents which might reverse hypertension related premature morbidity and mortality. The RAS system is an important modulator of blood pressure and vascular function/disease.

As noted above, in addition to an increase in the prevalence of salt sensitivity, an increased prevalence of reduced plasma renin levels have been noted in hypertensive African Americans and Caribbean Hispanics^[41-43]. Given the documented role of RAS in the progression of vascular disease, an attenuated risk of hypertension-related end-organ damage might also be expected in patients with low-renin hypertension. Paradoxically, however, many such individuals experience high rates of hypertension-related end-organ complications suggesting RAS may still be important at the tissue level in patients with reduced plasma renin levels.

Much of our understanding of the role of the RAS in blood-pressure regulation at a tissue level derives from Dahl salt-sensitive and salt-resistant rat studies as a model of human salt-sensitive, low renin hypertension that is more commonly noted in African Americans^[44]. Consumption of a high-salt diet by Dahl salt-sensitive rats results in hypertension and early onset of renal injury and dysfunction. This is associated with reduced plasma renin activity and angiotensinogen (ATG) concentrations, but accompanied by paradoxical elevations of renal tissue angiotensin (Ag) II, tissue Ag II receptor 1 (AT1) receptor expression and urinary ATG excretion as well as oxidative stress and activation of NAD(P)H oxidase in the kidney and cardiovascular tissues^[45,46]. These observations support a dissociation of low circulating RAS from the upregulated intrarenal and tissue RAS in this model. Despite the low circulating renin level, RAS blockade in Dahl salt-sensitive rats fed a high-salt diet reversed endothelial dysfunction, attenuated proteinuria and reduced cardio-renal injury even though it did not normalize the blood pressure supporting a blood pressure independent RAS related effect^[47]. These findings suggest that the intrarenal and tissue RAS may be more important in the pathogenesis of salt sensitive hypertension and hyper-

tensive nephropathy than the circulating RAS, which may be more reflective of the regulation of sodium balance, vascular resistance and arterial blood pressure.

The efficacy of RAS inhibition has also been shown to be widely effective in many other animal models of hypertension, including but not limited to hypertension in obese Zucker rats animals with renal mass reduction^[48,49] and rodents with hypertensive nephropathy induced by Ag II infusion^[50]. Osteopontin (OPN), which is a secreted matrix glycoprotein that is expressed in Ag II-injured tissues, is an important modulator of several of the Ag II-induced mechanisms of hypertensive nephropathy. Global deletion of OPN in hypertensive, albuminuric mice promoted Ag II-induced monocyte chemoattractant protein-1, NADPH oxidase subunits (NOX2, gp47phox and NOX4) and plasminogen activator inhibitor-1, compared to Ag II-infused wild-type mice^[50]. Also, inhibition of OPN expression may account for a mechanism by which Ag II blockade attenuated renal injury following renal ablation^[51], consistent with OPN modulating the effects of Ag I converting enzyme (ACE) inhibitor therapy in hypertensive nephropathy. Finally, Gonzalez-Villalobos *et al.*^[52] recently demonstrated that the absence of kidney ACE substantially blunts the hypertension induced by Ag II infusion or nitric oxide synthesis inhibition. Moreover, in mice that lack kidney ACE the renal responses to high serum Ag II such as intrarenal Ag II accumulation, sodium and water retention, and activation of transporter activating kinases Ste20-related proline alanine-rich kinase and oxidative stress response kinase were effectively prevented. These findings led them to conclude that renal ACE activity is required to increase local Ag II to stimulate sodium transport and induce hypertension^[52]. These findings are consistent with the importance of inhibition of intrarenal RAS to attenuate hypertension and its sequelae.

In summary, hypertension has been reported in animal models to induce glomerular hypertension and glomerular hyperfiltration, oxidative stress, inflammation, endothelial damage due to enhanced traffic of plasma proteins and/or increased translational and shear forces and other that may lead to worsening vascular disease, CKD and worsening blood pressure^[53,54]. Ag II is one of the more extensively studied mediators of vascular function. Ag II upregulates transforming growth factor- β 1, tumor necrosis factor- α , nuclear factor- κ B, OPN, several adhesion molecules and chemoattractants, and more recently, interactions with adiponectin and select microRNAs which together conspire to promote renal inflammation and fibrosis^[55,56]. The documented role of RAS in the progression of end organ damage has positioned it as a prime therapeutic target in high-risk patients. RAS blockade can reduce blood pressure, reverse endothelial dysfunction, attenuate proteinuria, and reduce renal injury independent of blood pressure changes^[47]. The paradoxical increase in tissue RAS in salt sensitive, low renin hypertension makes RAS blockade an important therapeutic option for treating African Americans and other patients

with hypertension and a circulating low renin profile^[44].

GENETIC POLYMORPHISMS, THE RAS, AND HYPERTENSION IN AFRICAN AMERICANS

At the level of the kidney the pool of intra-renal RAS is upregulated in CKD independently of systemic RAS. This pathological upregulation of the intra-renal RAS is marked by simultaneous increases in the AT1 expression and the number of the Ag II-producing cells, many of which are macrophages and serve as ectopic sources of angiotensin^[57]. In addition, the kidney not only contains ATG, angiotensin converting enzyme, and renin, but is a recipient of their physiological and pathophysiological actions^[58,59]. In CKD, AT1 receptor activation by Ag II raises superoxide production *via* upregulation of NAD(P)H oxidase, and inhibits Nrf2 expression, which is the master regulator of genes encoding many antioxidant and cytoprotective enzymes and related molecules^[60-62]. This may be an important mechanism of action through which intra-renal RAS promotes, oxidative stress, inflammation and subsequent tissue damage and dysfunction in animals, and likely humans with CKD and/or hypertension.

Several genetic variations (*e.g.*, promoter region variants of the *ATG* gene) have been identified which may contribute to ethnic disparities in salt-sensitive hypertension and response to RAS blockade. Tiago *et al.*^[63] reported a marked influence of homozygosity for the -20A allele ($n = 399$) of the ATG on the relationship between body mass index and systolic blood pressure ($r = 0.23$; $P < 0.0001$) in over 1000 South Africans of African ancestry. More specific to the response to RAS inhibition, the African-American Study of Kidney Disease and Hypertension (AASK) study showed that African Americans who were homozygous for the ACE polymorphism 12269G > A experienced a more rapid reduction in blood pressure following ACE inhibition than those who were heterozygous for this variant ($P < 0.001$), but blood pressure response to calcium channel blockers did not vary by ACE polymorphism variants^[64]. Similarly, ATG promoter region variants among a cohort of South Africans of African ancestry influenced the blood pressure response to an Angiotensin converting enzyme inhibitor (ACEI), but not to a calcium channel blocker^[65]. Recent genome-wide admixture mapping studies have demonstrated genetic variation in the regions of MYH9 and APOL 1 on chromosome 22 that have been estimated to explain over 50% of the difference in the rates of non-diabetic end-stage renal disease (ESRD) between white and black Americans^[13,66-69], but to date no reports have linked these gene variants to response to RAS inhibition therapy. Limited data exist for the study of ACE polymorphism variants in animal models of high BP. One report suggested a locus for the inducible, but not a constitutive, nitric oxide synthase cosegregated with

blood pressure in the Dahl salt-sensitive rat^[70], while microsatellite of ACE was reported to be associated with the development of salt-sensitive hypertension in the stroke-prone spontaneously hypertensive rat^[71].

TREATMENT TRIALS OF RAS INHIBITION IN AFRICAN AMERICANS

Most clinical trials of RAS inhibition as primary antihypertensive therapy in African Americans have been directed toward patients with diabetes, CKD, and/or high CVD risk. A summary of select trials of RAS inhibition as primary antihypertensive therapy in African Americans follows.

Diabetes

The Collaborative Study Group was the first major study to examine the efficacy of ACEI in slowing the progression of CKD in 409 participants with type 1 diabetes^[72], and while it demonstrated efficacy in comparison to usual care, the study included only 15 African Americans. Two subsequent major studies of RAS inhibition in persons with diabetic nephropathy, most of whom had hypertension, were the irbesartan (IDNT) and losartan (RENAAL) trials. These two trials both showed efficacy for ARB therapy and included higher proportions of ethnic minorities than most earlier studies with 13% African Americans and 5% Hispanics in the former and 15% African Americans and 18% Hispanics in the latter^[73,74]. Although not powered to perform subgroup analyses according to ethnicity, these studies strongly suggest that the positive outcomes of RAS inhibition extended to all study participants. Moreover, a post-hoc analysis of RENAAL found no ethnic differences in the relationship of baseline albuminuria or 6-mo antiproteinuric response to therapy to ESRD risk, or the overall renoprotective effect of ARB therapy (1513 participants followed for 3.4 years with final SBP of 141 mmHg)^[75].

CKD

The AASK is the largest prospective CKD study to focus on African Americans to date^[76,77]. The AASK trial ($n = 1094$) was a randomized controlled study that examined the effects of three classes of initial antihypertensive therapy (ACEI, β -blocker or calcium channel blocker) and two levels of blood pressure control: intensive ($\leq 120/80$ mmHg) and standard (approximately 135-140/85-90 mmHg) on the progression of renal function and clinical outcomes in a high-risk cohort with hypertension-related CKD^[78]. Diuretics were not among the three randomized classes of antihypertensive agents as it was assumed the majority of study participants would require diuretic therapy due to their impaired renal function and associated volume retention and therefore the majority of study participants would require diuretics, allowing the design to most closely emulate clinical practice. Indeed, nearly 90% of AASK participants re-

quired adjunct diuretic therapy to achieve target blood pressure levels. While calcium channel blockers were the most commonly prescribed antihypertensive for African Americans with CKD due to their blood pressure lowering efficacy, the calcium channel blocker arm of AASK was terminated early because of increased rates of adverse clinical events^[76]. AASK demonstrated that clinical cardio-renal outcomes in African Americans: were improved with ACEI in comparison to β -blocker or calcium channel blocker, with diuretics and other agents added as needed^[79]. While outcomes did not initially differ between intensive ($\leq 120/80$ mmHg) and standard (approximately 135-140/85-90 mmHg) BP targets^[79], longer term follow up (8.8 to 12.2 years) in the intensive control group (mean blood pressure was 130/78 mmHg) compared to standard care (141/86 mmHg) revealed a benefit in patients with baseline protein-to-creatinine ratio > 0.22 (equivalent to baseline protein excretion of > 300 mg/d), but not the overall cohort^[80].

Importantly, AASK demonstrated that blood pressure can be controlled in African Americans with CKD and that combined clinical outcomes (cardiovascular and renal) can be improved by using not only a beta blocker or calcium channel blocker but an ACEI as initial therapy to reach a usual or strict blood-pressure target, with diuretics in most, and other agents added as needed^[71], and that ACEI therapy led to the best clinical outcomes^[79]. This contrasts previous^[7] and more recent suggestions^[3] that RAS inhibition is of limited benefit in African Americans. The notion of the overall efficacy of RAS inhibition is further supported by a recent meta-analysis of 25 randomized controlled trials ($n = 45758$) by Balamuthusamy *et al*^[81] who found improved or equivalent CVD outcomes in patients with diabetic or non-diabetic CKD and proteinuria treated with RAS blockade (ACEI/ARB) in comparison to placebo and control (β -blocker, calcium-channel blockers and other antihypertensive-based therapy). While the ethnic composition of the meta-analysis was not provided, the preponderance of evidence supports the important role for RAS blockade in treating patients with CKD and proteinuria, including African Americans. Secondary analysis of Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) trial, detailed below, showed that in patients with reduced renal function, RAS inhibition with lisinopril was equally as effective as amlodipine and chlorthalidone in reducing the rate of development of ESRD^[82].

HIGH CARDIOVASCULAR RISK

The Heart Outcomes Prevention Evaluation trial assessed the effectiveness of RAS inhibition in nearly 10000 high-risk patients (55 years of age or older) who had evidence of vascular disease or diabetes plus one other cardiovascular risk factor, but no evidence of over health failure^[83]. They found ramipril significantly reduced the rates of death, myocardial infarction, and stroke^[83]. Unfortunately, the racial/ethnic composition of the study cohort was

not described. Another key hypertension trial, which included ACEI therapy and a large percentage of minority participants, was the ALLHAT. ALLHAT enrolled nearly 34000 high-risk hypertensive patients, of whom 32% were black and 16% were Hispanic^[84]. ALLHAT analysis found that first-line therapy with chlorthalidone, amlodipine or lisinopril were similar in efficacy for preventing cardiovascular events^[85]. First-line therapy with alpha blockade was not as effective and the alpha blocker arm was discontinued early^[86]. Subgroup analysis by race/ethnicity revealed no significant differences by class of therapy from that of the overall trial results. Unfortunately the second line drug for both amlodipine and lisinopril could not be a diuretic or a complementary RAS inhibitor or calcium channel blocker, respectively, so the design limited the ability of ALLHAT to test common clinical practice and practice guidelines. This is especially important for African Americans with high blood pressure who are more likely to require 2 or 3 drugs to achieve blood pressure goal and especially important for RAS inhibition which has been most effective in combination with a diuretic when a second agent is needed^[79]. Many authorities still favor initial therapy with RAS blockade, especially in patients with hypertension complicated by diabetes, CKD, or CVD where diuretics are commonly included in treatment^[1,2,87]. Secondary analysis of ALLHAT showed that in patients with reduced renal function, RAS inhibition with lisinopril was equally as effective as amlodipine and chlorthalidone in reducing the rate of development of ESRD^[82].

SPECIAL CONSIDERATIONS FOR THE USE OF ACEI IN TREATING HYPERTENSION IN AFRICAN AMERICANS

Consideration is necessary of the well-recognized common effects noted with some agents that inhibit the RAS system. ACEI related adverse events are relatively common in African Americans and Chinese^[88-90]. Of note, the rate of angioedema in blacks is three times that of non-blacks^[88,89], and the rate of ACEI discontinuation due to cough is also very high^[91]. Possible mechanisms which could account for the increased incidence of ACEI-related adverse effects in African Americans are angiotensin-converting enzyme and bradykinin gene polymorphisms as have been demonstrated in East Asians^[92]. Also notable is that the initiation of RAS inhibition therapy can lead to an acute reduction in renal function regardless of racial/ethnic background, especially in patients with advanced CKD. In most instances this reduction in glomerular filtration rate is a potentially reversible physiologic hemodynamic effect and a modest initial fall in renal function may be a predictor of long-term renoprotection^[93,94]. However, close care is required to avoid complications such as hyperkalemia or hypotension, which may occur in some patients with deteriorating renal func-

tion and would warrant discontinuation.

The optimism for enhanced efficacy of RAS inhibition by using a combination of ARB/ACE inhibitor has recently dampened. The ONTARGET trial followed 25000 participants (11% Aboriginal/African) with diabetes with end-organ damage (13% with microalbuminuria) or vascular disease for 4.5 years, randomized to Ramipril group (ACE inhibitor), Telmisartan group (ARB), or both^[95]. They found no difference in the composite outcome of cardiovascular events including death or hospitalization for heart failure between groups, and at trend toward increased cardiovascular events in the group receiving combination ARB/ACEI^[95].

TARGET BLOOD PRESSURE IN AFRICAN AMERICANS

The 2007 European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) Guidelines recommended two distinct BP targets: 140/90 mmHg in low-moderate risk hypertensive individuals and 130/80 mmHg in high-risk hypertensive persons (*e.g.*, those with diabetes, cerebrovascular, cardiovascular, or renal disease)^[96]. The 2013 ESH and the ESC Guidelines recommend a blood pressure target of 140/90 mmHg regardless of risk, with a less stringent SBP goal of between 150 and 140 mmHg in the elderly^[2]. They found no evidence to support a lower blood pressure goal (130/80 mmHg) in patients with diabetes or a history of cardiovascular or renal disease^[2]. This was not specific to any racial/ethnic group. This lack of support for a lower blood pressure goal is further supported by the results of the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial^[97]. The ACCORD trial, which included 19.3% black participants with type 2 DM, used both ACEI and ARB as part of antihypertensive therapeutic approach and found no benefit in regards to major cardiovascular events with a SBP target of < 120 *vs* < 140 mmHg. This is also consistent with the findings mentioned earlier from the AASK trial, which found no differences in clinical outcomes between intensive (\leq 120/80 mmHg) and standard (approximately 135-140/85-90 mmHg) blood pressure targets^[79]. However, the trend toward improved outcomes at a lower blood pressure in patients with elevated baseline protein-to-creatinine ratio > 0.22 (equivalent to baseline protein excretion of > 300 mg/d)^[80], suggests further studies are needed to assess the benefit of a lower target blood pressure in higher risk groups such as African Americans with target organ damage. In fact, based on this and other secondary analyses, the International Society on Hypertension in Blacks consensus statement suggested a BP target of < 130/80 mmHg in hypertensive blacks with target organ damage^[87], although others suggest the data to support such a recommendation is still insufficient^[98].

One of the obstacles to attaining target blood pressure goals in African Americans is the issue of medication adherence. Low medication adherence rates and

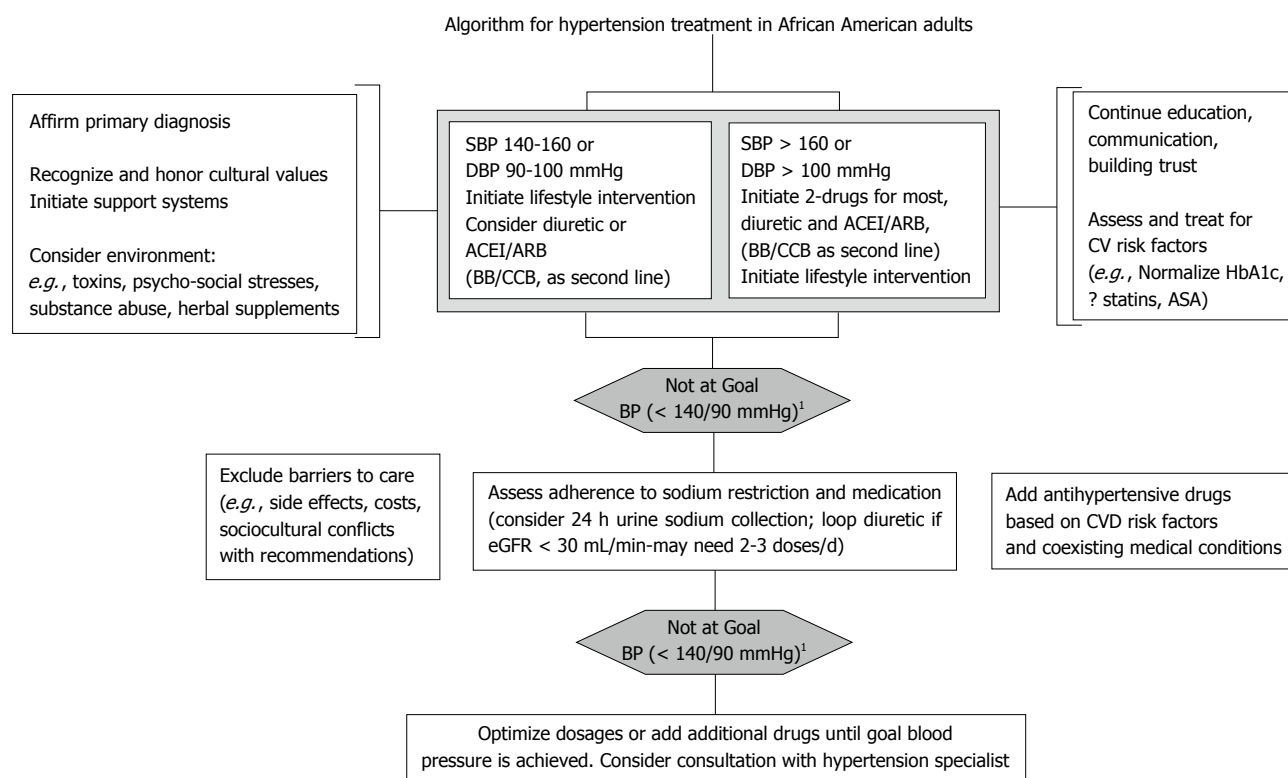


Figure 1 Algorithm for hypertension treatment in African American adults (adapted from ref. [86]). ¹For persons > 60 years of age consider < 150/100 mmHg if initial SBP is > 160 mmHg^[2]. ACEI: Angiotensin converting enzyme inhibitor; ARB: Angiotensin receptor blocker; BB: Beta blocker; CCB: Calcium channel blocker; SBP: Systolic blood pressure; CVD: Cardiovascular disease; DBP: Diastolic blood pressure; eGFR: Estimated glomerular filtration rate; CV: Cardiovascular; ASA: Aspirin; ?: Possibly the use of statins or aspirin or possible use of statins or aspirin; BP: Blood pressure.

higher rates of uncontrolled blood pressures are more common in African Americans^[33,99,100]. Keys to the effectiveness of ACEI therapy is adherence to both pharmacologic and non-pharmacologic therapy particularly in African Americans whose lower adherence rates have been attributable to both patient-related and physician-related factors including medication cost, insurance issues and access to healthcare. Aggressive measures are required to target interventions such as patient education focused on patient misconceptions regarding hypertension, home visits by trained community health workers, culturally appropriate storytelling, home blood pressure monitoring and behavioral counseling—all of which have been associated with improved medication adherence and decreased blood pressure measurements in blacks^[100,101].

CONCLUSION

Health care providers currently consider a patient's age, gender and ethnic background when making clinical decisions based on the evidence from clinical research findings. As in the non-African American hypertensive patient, first excluding primary renal or secondary causes of hypertension, then establishing a comprehensive treatment approach, is paramount to slowing the progression of end organ damage (Figure 1). Aggressive treatment of the primary etiology, addressing select lifestyle and socio-cultural issues, and the use of two or more antihyperten-

sive agents for control of blood pressure is typically required in African American patients^[102]. The existing data highly supports the inclusion of RAS blockade agents as initial therapy for African Americans with hypertension. In fact, the data from animal models of salt sensitive, low renin hypertension suggest RAS blockade may be even more imperative in treating African Americans with hypertension than the general population. These agents appear to confer additional end organ protection beyond that offered by other antihypertensive agents in this patient subgroup. Importantly, there is no evidence of reduced efficacy for ACEI or ARB therapy on clinical outcomes in African Americans^[103].

In conclusion, the overall treatment plan should be guided by individual patient response, coexisting risk factors and potential sociocultural considerations such as cost of medications and insurance coverage, which affect adherence to both pharmacologic and non-pharmacologic interventions^[14]. In all racial groups, blood pressure target goals in uncomplicated hypertension based on clinical trial data is < 140/90 mmHg with debate over a lower target (< 130/80 mmHg) in cases with end organ damage due to the data mostly being surrogate markers with a lack of consistent hard outcome data. Further elucidation of the optimal treatment for hypertension may be provided by the ongoing NIH-funded Systolic Blood Pressure Intervention Trial trial which will include a diverse patient population in regards to gender, race/ethnicity

and comorbidities in over 7500 persons over 55 years of age^[104].

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Metabolic syndrome in hypertensive patients: An unholy alliance

Giuseppe Mulè, Ilenia Calcaterra, Emilio Nardi, Giovanni Cerasola, Santina Cottone

Giuseppe Mulè, Ilenia Calcaterra, Emilio Nardi, Giovanni Cerasola, Santina Cottone, Dipartimento Biomedico di Medicina Interna e Specialistica (DIBIMIS), Unit of Nephrology and Hypertension, European Society of Hypertension Excellence Centre, Università di Palermo, 90146 Palermo, Italy

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Correspondence to: Giuseppe Mulè, MD, Dipartimento Biomedico di Medicina Interna e Specialistica (DIBIMIS), Unit of Nephrology and Hypertension, European Society of Hypertension Excellence Centre, Università di Palermo, Via Monte San Calogero 129, 90146 Palermo, Italy. giuseppe.mule@unipa.it

Telephone: +39-091-6554578 Fax: +39-091-6554338

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showed that the MetS, even without type 2 diabetes, confers an increased risk of cardiovascular morbidity and mortality in different populations including also hypertensive patients. It is likely that the enhanced cardiovascular risk associated with MetS in patients with high blood pressure may be largely mediated through an increased prevalence of preclinical cardiovascular and renal changes, such as left ventricular hypertrophy, early carotid atherosclerosis, impaired aortic elasticity, hypertensive retinopathy and microalbuminuria. Indeed, many reports support this notion, showing that hypertensive patients with MetS exhibit, more often than those without it, these early signs of end organ damage, most of which are recognized as significant independent predictors of adverse cardiovascular outcomes.

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Key words: Arterial hypertension; Metabolic syndrome; Target organ damage; Cardiovascular risk

Abstract

For many years, it has been recognized that hypertension tends to cluster with various anthropometric and metabolic abnormalities including abdominal obesity, elevated triglycerides, reduced high-density lipoprotein cholesterol, glucose intolerance, insulin resistance and hyperuricemia. This constellation of various conditions has been transformed from a pathophysiological concept to a clinical entity, which has been defined metabolic syndrome (MetS). The consequences of the MetS have been difficult to assess without commonly accepted criteria to diagnose it. For this reason, on 2009 the International Diabetes Federation, the American Heart Association and other scientific organizations proposed a unified MetS definition. The incidence of the MetS has been increasing worldwide in parallel with an increase in overweight and obesity. The epidemic proportion reached by the MetS represents a major public health challenge, because several lines of evidence

Core tip: Several lines of evidence suggest that metabolic syndrome (MetS) may amplify hypertension-related target organ damage (TOD). Some of MetS components, when considered individually may have little or no influence on TOD, but when taken together may synergistically interact promoting the development of left ventricular hypertrophy, aortic stiffness and microalbuminuria. The marked tendency of hypertensive patients with MetS to develop these manifestations of subclinical organ damage, that are well-known predictors of cardiovascular events, largely explain the increased morbidity and mortality associated with the syndrome. Therefore, identifying MetS in hypertensive patients may enable the clinician to better assess the cardiovascular risk.

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INTRODUCTION

For many years, it has been recognized that high blood pressure is often associated with various anthropometric and metabolic abnormalities including abdominal obesity, elevated triglycerides, reduced high-density lipoprotein (HDL) cholesterol, glucose intolerance and insulin resistance.

Several lines of evidence support the notion that these traits occur simultaneously to a greater degree than would be expected by chance alone. This evidence supports the existence of a discrete disorder meriting in the appellation as a “metabolic syndrome”. A variety of clinical and biohumoral alterations may co-exist with the main components of the metabolic syndrome: hyperuricemia, increases in apolipoprotein B and small dense low-density lipoprotein cholesterol, prothrombotic factors, chronic low grade inflammation, non-alcoholic fatty liver disease and/or non-alcoholic steatohepatitis, obstructive sleep apnoea and polycystic ovarian disease. Many of these conditions may contribute to explain why the metabolic syndrome (MetS) conveys an increased risk of developing subclinical and overt cardiovascular and renal diseases.

METABOLIC SYNDROME DEFINITIONS

In the effort to introduce the MetS into clinical practice, several scientific organizations have attempted to formulate working definition of the syndrome. The first proposals came in 1998^[1] and in 1999^[2] from a consultation group on the definition of diabetes for the World Health Organization (WHO)^[2]. Alternative definitions have been proposed subsequently by the European Group for the Study of Insulin Resistance (EGIR)^[3], the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III)^[4] the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI)^[5], the American Society of Clinical Endocrinologists (AACE)^[6] and the International Diabetes Federation (IDF)^[7]. Recently, IDF, AHA/NHLBI and other scientific societies, in an attempt to unify discordant criteria between previous definitions of MetS, proposed a new “harmonizing” definition of this syndrome^[8]. All definitions include a measure of blood pressure (BP), triglycerides, HDL cholesterol, and fasting glucose. They differ with respect to the selection of cutoff points and a measure of obesity. In contrast to the glucocentric approach of the WHO and EGIR definitions, in which the presence of insulin resistance is the starting

point, the ATP III definition is based on the number of abnormalities only, whereas the AACE definition considers the number of abnormalities in selected subjects with high risk of insulin resistance. The ATP III and WHO definitions implicitly include type 2 diabetes (T2DM) as syndrome traits. Not all experts agree that T2DM should be part of the definition, as the importance of the syndrome is that it identifies patients at increased risk for the development of diabetes^[8,9].

Among the various definitions proposed the most widely used is that of the ATP III or the AHA/NHLBI version, that slightly revised the former by lowering the threshold for fasting glucose from 110 to 100 mg/dL.

The wide use of these definitions is due primarily because they provide a relatively simple approach for diagnosing MetS by employing easily measurable risk factors.

In the revised ATP III definition^[5], MetS is diagnosed when at least three or more of the following abnormalities are present: BP $\geq 130/85$ mmHg (or drug treatment for hypertension), HDL < 1.0 mmol/L (40 mg/dL) in men or < 1.3 mmol/L (50 mg/dL) in women (or drug treatment for reduced HDL); fasting glucose ≥ 5.6 mmol/L (100 mg/dL) (or drug treatment for elevated glucose); triglycerides > 1.7 mmol/L (150 mg/dL) (or drug treatment for elevated triglycerides); and waist circumference > 102 cm in men or > 88 cm in women^[5].

On 2005 the IDF has proposed a set of criteria that are similar to those of the updated ATP III criteria. In fact, thresholds are identical for triglycerides, HDL-C, BP, and plasma glucose. The major difference is that the IDF considered central obesity, as assessed by waist circumference (WC), essential for diagnosis. Moreover, in this obesity-centric definition the WC cutoffs were adjusted for different ethnic groups, taking into account that the risk associated with a particular waist measurement (especially for diabetes development) will differ in different populations. But despite the attempt to standardize clinical definition of MetS, this has led to some confusion on the part of clinicians regarding how to identify patients with the syndrome.

Thus came the initiative of the IDF and the AHA/NHLBI, joined by the World Heart Federation, International Atherosclerosis Society, and International Association for the Study of Obesity^[8], to develop one unified definition.

The main difference between the ATP III and the IDF diagnostic criteria is that in the IDF definition abdominal obesity is a prerequisite of the diagnosis of MetS. As a major step in consensus, this obligation has been reversed; therefore the “harmonizing” definition is identical to the revised ATP III definition except that IDF waist circumference cut points were used^[8]. In Europeans (white people of European origin, regardless of where they live in the world) WC thresholds were the same as that used by the EGIR (≥ 94 cm for males and ≥ 80 cm for females), and lower than the ATP III recommendations^[7]. For the Asian people IDF adopted even lower cutpoints for men (≥ 90 cm) and the same as for Europ-

ids for women.

Similarly to adults, no general consensus exists regarding the definition of MetS in children and adolescents^[9,10]. Furthermore, studies published so far have used their own set of variables, number of criteria (three or four) and different cut-off points to define risk factors associated with MetS. In 2007, a consensus report was published by the IDF group^[10], including three age groups: 6 to < 10, 10 to < 16 and 16 + years (adult criteria).

Based on this report, obesity is defined as WC \geq 90th percentile, or adult cut-off if lower, while all other parameters are defined based, rather than percentiles, on absolute numbers, that are the same used for adults^[10].

A scientific statement from the AHA and other scientific societies, published on 2009, called attention to the fact that, especially during adolescence, a marked instability exists in the categorical diagnosis of MetS. This instability, which includes both gain and loss of the diagnosis, suggests that the syndrome has reduced clinical utility in adolescence^[9].

IS METABOLIC SYNDROME REALLY A SYNDROME?

Some controversy also exists about whether the MetS is a true syndrome or a mixture of unrelated phenotypes.

Two major health organizations in Europe (the European Association for the Study of Diabetes) and the United States (American Diabetes Association) have claimed that the MetS is not a single pathophysiological entity, that its identification has no clinical utility, and that clinical emphasis should rather be placed on effectively treating any cardiovascular (CV) risk factor that is present^[11,12]. We believe, together with many experts on CV risk^[8,13,14], that this clustering of interrelated metabolic risk factors is a useful construct, and although it needs to be better defined, represents a good basis for calling this as a syndrome. The rationale supporting use of the MetS includes the following: (1) the label of MetS seems to be an important way to educate patients about the connection between their lifestyle, health risks, and medical outcomes; (2) it provides a framework for research exploring a possible unifying pathophysiological basis for the observed cluster of risk factors; (3) it quantifies chronic disease risk within populations and facilitates between-country comparisons; (4) it can guide relative risk prediction and clinical management decisions; (5) it results from the association of individuals components that are often defined by values that are lower than those meeting the definition of risk factors by many guidelines, which may thus fail to detect the presence of a high CV risk in several subjects with MetS; and (6) it provides an easily comprehensible public health message and reminds health professionals of the need to assess related risk factors when one risk factor is detected ultimately helping implementation of CV prevention^[8,9].

On the other hand, the criteria used to diagnose the

MetS have major limitations including: the dichotomization of risk factors; the attribution of relative as opposed to absolute risk; the differing predictive value of risk factor combinations; the inclusion of individuals with established diabetes and heart disease^[9,10].

PREVALENCE OF METABOLIC SYNDROME IN GENERAL POPULATION AND IN HYPERTENSIVE PATIENTS

The prevalence of the MetS is at least in part dependent on the definition of the syndrome and its components and on the composition (sex, age, race, and ethnicity) of the population studied^[14-27]. However, there is a strong epidemiological evidence that, regardless of the criteria used, the prevalence of MetS is high and rising in all western society and in Asia, very likely as a result of obesity epidemic^[14-20]. In general, it has been estimated that approximately 10%-30% of the world's adult population has the MetS^[14]. A very consistent finding in all of these studies is that the prevalence of the MetS is highly age-dependent^[14-19]. Data regarding gender effect on MetS prevalence are conflicting with the majority of the studies finding the highest prevalence in women compared to men^[14-19]. The conflicting results with respect to gender effect may partly be explained by the application of different definitions for the MetS. The application of the modified WHO criteria tends to increase the prevalence of MetS in men^[18,19].

Since high BP is a key component of MetS, it is not surprising that in MetS patients arterial hypertension is highly prevalent. The Pressioni Arteriose Monitorate E Loro Associazioni (PAMELA) population study revealed that high normal BP values and hypertension were present in 80% of individuals with MetS^[21]. Conversely, the prevalence of MetS is more elevated in hypertensive patients than in general population^[18-20,22-36].

In a large French population, the prevalence of MetS was 5.4% ($n = 1181$) among normotensive men and 2.8% ($n = 360$) among normotensive women, and rose to 19.3% ($n = 3490$) for hypertensive men and 14.8% ($n = 1200$) for hypertensive women. Much higher prevalences were reported in other studies performed only in hypertensive patients^[23-36].

In the Progetto Ipertensione Umbria Monitoraggio Ambulatoriale (PIUMA) study, a prospective observational investigation of 1742 Italian adult subjects with essential hypertension, MetS, defined according to ATP III criteria, was diagnosed in 34% of the population^[25].

Similar data were obtained in our cross-sectional study conducted in 353 essential hypertensives and 37% of whom had MetS^[26]. In our study population, prevalence of MetS was higher in women than it was in men. This greater proportion of women with MetS was explained by a higher prevalence of visceral obesity and of low HDL values in females when compared to males^[26].

An even greater prevalence of MetS was observed

Prohypertensive mechanisms in metabolic syndrome

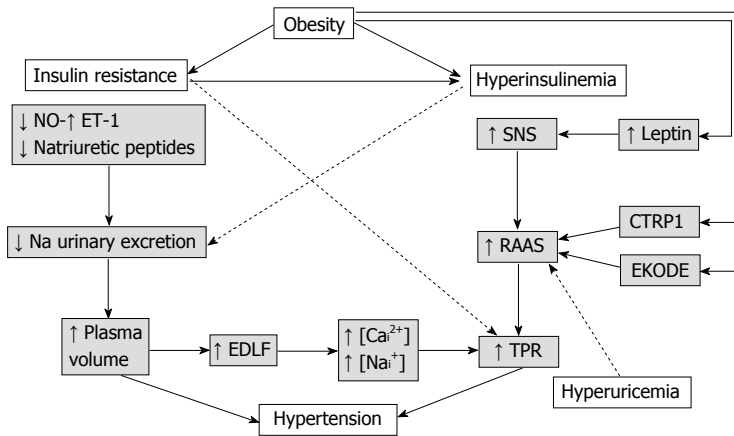


Figure 1 Hypothetical mechanisms by which the metabolic syndrome may lead to high blood pressure. NO: Nitric oxide; ET-1: Endothelin-1; SNS: Sympathetic nervous system; RAAS: Renin-angiotensin-aldosterone system; CTRP1: Complement-C1q tumor necrosis factor-related protein 1; EKODE: Epoxy-keno derivative of linoleic acid; TPR: Total peripheral resistance; EDLF: Endogenous digoxin-like factor; $[Ca^{2+}]$: Intracellular concentration of calcium; $[Na^{+}]$: Intracellular concentration of sodium.

in the Global Cardiometabolic Risk Profile in Patients with hypertension disease (GOOD) study^[33]. This was an observational, cross-sectional survey conducted in 305 sites in 12 European countries. Among the 3370 outpatients included in the analyses 58% had the MetS. This very high prevalence is probably explained by the older age (61 years) of the study population when compared to those of the other investigations conducted in hypertensive subjects. In the same survey it was noticed that the proportion of patients with uncontrolled BP was significantly higher among the subjects with MetS compared with those without it ($P < 0.001$)^[33]. Analogous results were found among the hypertensive population of the Korean National Health and Nutrition Examination Survey^[34] and in the Renal Dysfunction in Hypertension (REDHY) study. In the latter investigation, where a total of 1856 Sicilian hypertensive individuals, free from diabetes mellitus were enrolled^[35], a significantly higher ($P < 0.001$) percentage of patients with uncontrolled BP ($> 140/90$ mmHg) were found in the group with MetS (79%) as compared to the subjects without MetS (71%).

It has been also reported a high frequency of resistant hypertension among individuals with MetS^[36], that can be attributed to a number of pathophysiological mechanisms^[36] that will be described in the following section.

The prevalence of the MetS is growing worldwide^[14-17]. Between 2008 and 2010, the proportion of the hypertensive population with MetS was forecast to increase to 78%, 45% and 43% in Germany, Spain and Italy respectively^[16]. All MetS components were forecast to rise with the prevalence of abdominal obesity and impaired fasting glucose increasing the most. Total annual costs of hypertension with MetS amounted to €24427, €1909 and €4877 million respectively in Germany, Spain and Italy in 2008. By 2020, keeping costs set at 2008 prices, these annual costs of hypertension with MetS were forecast to rise by 59%, 179%, 157% in Germany, Spain and Italy

respectively. The largest component of the total annual economic burden of hypertensive patients with MetS was the treatment and management of the consequence of disease rather than the management of hypertension itself including physician and drug costs^[16].

Pathophysiology of hypertension in metabolic syndrome

Several mechanisms have been hypothesized to explain why the MetS may be considered as a prohypertensive state^[32] (Figure 1).

Although further research is required to better understand the pathophysiology behind the syndrome and the gene-environment interactions that increase susceptibility, there is general agreement that visceral obesity and insulin resistance (IR) are at the core of most cases of MetS^[1-6,37,38]. It is widely believed that IR results from a combination of genetic and environmental factors^[1-6]. Resistance to insulin-mediated glucose disposal determines a compensatory hyperinsulinemia, which serves to maintain glucose homeostasis. However, this initially adaptive mechanism ultimately may promote hypertension and various atherogenic processes. It is only after the pancreas is unable to meet the increased demand for insulin necessary to overcome IR that glucose control becomes abnormal. Therefore, hyperglycemia signifies a more advanced stage in the loss of normal glucose homeostasis^[1-6].

About 50% of patients with essential hypertension are insulin-resistant^[18-20,39-41]. Independently of body mass index, hypertensive individuals, when compared with healthy normotensive controls, have higher fasting and postprandial insulin levels, and greater reductions in insulin sensitivity^[39-42].

Insulin, in response to states of over-nutrition, stimulates the sympathetic nervous system (SNS) to promote thermogenesis and to minimize weight gain. The insulin-mediated hyperadrenergic state, however, leads to an increase in heart rate, and BP^[37,43,44].

Other factors may contribute to the sympathetic activation occurring in MetS. They include leptin, which increases in obesity and has been shown to act as a powerful sympathostimulator^[36,43-46]. Sleep apnoea, which frequently occurs in obesity, may also play a role because its sympathoexcitatory effect *via* the hypoxic activation of the chemoreceptor reflex^[44,46,47].

The enhanced SNS activity and insulin and leptin *per se*^[48] stimulate renal sodium absorption leading to volume expansion and further elevation of BP^[36,43-46] (Figure 1).

Moreover, insulin can cause an upregulation of angiotensin II type I receptors by post-transcriptional mechanisms such as stabilization of receptor mRNA and prolongation of its half life^[49]. The increase in angiotensin II type I receptors potentiates the physiologic actions of angiotensin II that include peripheral vasoconstriction and plasma volume expansion. Furthermore, overexpression of systemic as well as local adipose tissue renin-angiotensin-aldosterone system (RAAS) has been documented in obese persons^[36,45,46,50,51].

The increased activity of RAAS in subjects with MetS may be related also to vitamin D deficiency. Indeed, vitamin D status has been inversely associated with MetS^[52,53].

Experimental studies have suggested that vitamin D may exert its beneficial effects by stimulating the expression of insulin receptor to improve insulin responsiveness for glucose transport or by controlling calcium influx, which is essential for the insulin mediated intracellular process in insulin responsive tissues^[53].

In some studies, increased plasma aldosterone concentrations (PAC) have been reported in obese subjects. These elevated aldosterone levels are often out of proportion to the increase in renin activity^[46,54-58]. Indeed, it has been demonstrated that a variety of adipose tissue-derived factors can stimulate aldosterone synthesis^[36,46,55,57]. Goodfriend *et al*^[55] reported that an epoxy-keto derivative of linoleic acid (EKODE), one of the oxidized products of fatty acids, stimulates aldosterone secretion in rat adrenal cells. More recently, *in vitro* experiments documented that human adipocytes secrete potent mineralocorticoid releasing factors. Among these a Complement-C1q tumor necrosis factor-related protein 1 (CTRP1) is able to increase aldosterone production in cultured human adrenal cortical cells, and serum CTRP1 expression was higher in a small number of hypertensive patients compared with healthy volunteers^[57,58].

On the other hand, the low levels of plasma natriuretic peptides observed in individuals with obesity and MetS might predispose to increased adrenal production of aldosterone, because the stimulatory effect of EKODE on aldosteronogenesis is inhibited by natriuretic peptides^[57]. Another putative mechanism explaining augmented PAC may be endothelin 1^[59-61], which is increased in insulin resistance states^[59-61].

Moreover, insulin resistance and the accompanying compensatory hyperinsulinemia may contribute toward increasing PAC, because insulin is known to stimulate aldosterone synthesis *in vitro*^[62] and reciprocal relation-

ships between aldosterone, insulin resistance and hyperinsulinemia have been described in clinical studies^[36,63,64] (Figure 1).

Adipocytes appear to have all the components of the RAAS and thus may produce locally generated angiotensin II and aldosterone^[36,46,50,51]. On the other hand, increased intrarenal pressure accompanying perirenal fat deposition in obesity contributes to the increased activity of RAAS^[45].

Insulin resistance/hyperinsulinemia and visceral obesity appear to predispose patients to impaired peripheral glucose utilization and nitric oxide (NO) production^[65,66]. Indeed, insulin is a mediator of important vasodilatory functions on the vasculature. In obese individuals with insulin resistance, these functions are lost or even reversed leading to impaired vascular relaxation and hypertension^[65,66]. The underlying mechanism may be an impairment of NO-mediated vasodilation and a relative increase in the activity of endothelins^[59,60,65,66]. Cellular response to insulin is mediated by means of 2 pathways: phosphatidylinositol (PI3) 3-kinase and mitogen-activated protein (MAP) kinase^[65,66]. Activation of the PI3 kinase pathway is associated with the metabolic effects of insulin, including glucose transport, and NO-synthesis, whereas MAP kinase activation is associated with mitogenic effects, such as cell growth and proliferation. It has been demonstrated that in the setting of insulin resistance or T2DM, insulin had reduced effects on PI3 kinase-mediated pathways, while maintaining MAP kinase activity^[65,66].

Furthermore, the increased peripheral vascular resistance that often accompanies insulin resistance may be due in part to altered divalent cation metabolism ("cation imbalance") of vascular smooth muscle cells (VSMC)^[39,67]. One mechanism by which insulin, and its homologous peptide insulin-like growth factor-1 (IGF-1), attenuate vascular contractility is through effects on VSMC divalent cation metabolism^[67]. These hormones reduce Ca^{2+} influx into VSMCs in conjunction with reductions in VSMC contractile responses. It is thought that the mechanism by which insulin and IGF-1 decreases VSMC intracellular Ca^{2+} vasoconstriction is through stimulation of the Na^+/K^+ -ATPase pump^[67]. It has been demonstrated that insulin/IGF-1 activation of the PI3-kinase pathway is critical for the ability of these peptides to stimulate the pump^[67]. Thus, altered PI3-kinase responses to insulin/IGF-1, described in insulin resistance states, may explain the decreased ability of those peptides to mediate vasodilation in insulin-resistant patients^[67]. As angiotensin II has been shown to interfere with PI3 activation in VSMC and cardiomyocytes, overexpression of the tissue RAAS may be one of the major factors in cardiovascular insulin/IGF-1 resistance^[50,51,67,68].

Other factors may contribute to reduce the activity of Na^+/K^+ -ATPase pump in patients with MetS, such as the increased production of the endogenous digoxin-like factor^[68,69]. Elevated plasma levels of this substance have been documented in obese hypertensives with glucose intolerance^[69] and in several circumstances characterized

by volume expansion^[68]. This Na^+/K^+ -ATPase inhibitor promotes natriuresis but also produces accumulation of intracellular sodium, reducing in turn the sodium-calcium exchange system, and increasing cytosolic free calcium^[68]. The cation imbalance may lead to enhanced VSMC contraction and to an elevation of peripheral vascular resistance. Moreover, reducing the sodium pump activity may exaggerate neural stimulation and norepinephrine overflow, which might contribute to increase BP^[68].

On the other hand, the low levels of plasma natriuretic peptides observed in obese and overweight individuals, especially in those with IR^[70] may also predispose to salt retention and increased activation of the sympathetic and renin-angiotensin systems, leading to persistent BP elevations in patients with MetS^[36,46,70] (Figure 1).

Patients with MetS have often raised levels of serum uric acid (SUA)^[6,71]. Hyperuricemia has been usually attributed to hyperinsulinemia and IR in MetS^[6] and is not acknowledged as a main mediator of MetS, and CV diseases (CVD) development. However, investigations conducted in the last decades have changed this traditional view, supporting the concept of an independent link between hyperuricemia and increased risk of MetS, diabetes, hypertension, kidney disease and CV disorders^[71-74]. Pharmacologically induced mild-to-moderate hyperuricemia, *via* oxonic acid administration, in rats resulted in the development of high BP^[72,73]. Experimental studies suggest that SUA might play a role in initiating hypertension through multiple mechanisms, including induction of oxidative stress, activation of RAAS and inhibition of NO^[72]. A plausible common pathway for the above mechanisms is the development of renal arteriolar disease with interstitial macrophage and T-cell infiltration, eventually leading to renal vasoconstriction and ischemia^[72]. Subsequent studies showed that the hypertension developed in 2 phases. Initially, reducing SUA with either xanthine oxidase inhibitors or uricosuric agents could directly reverse the hypertension. Hypertension during this (salt-resistant) phase was mediated by uric acid-dependent activation of the RAAS, by the induction of oxidative stress, and by the reduction in endothelial NO levels^[72]. Over time the animals developed significant renal microvascular disease and tubulointerstitial inflammation, and the hypertension became kidney-dependent and salt-sensitive and persisted despite allowing uric acid levels to return to baseline levels^[73].

Lowering SUA with either allopurinol or probenecid has been shown to markedly reduce BP in pilot studies of adolescents with hypertension or prehypertension, whereas effects on adults with primary hypertension are less prominent^[72,73]. More recently, in 2045 participants of the PAMELA study, elevated SUA levels predicted new-onset home and ambulatory hypertension as well as cardiovascular and all-cause mortality^[74]. There are also studies that suggest SUA may not play a role in hypertension and related disorders^[73,75,76]. One of the strongest

arguments is based on gene wide association studies, which have been able to link polymorphisms in urate transporters with hyperuricemia and gout but not with high BP^[73]. Therefore, despite the findings obtained in animals studies and in adolescents, the question regarding the exact role of uric acid in inducing hypertension and CV diseases remains unanswered.

All the above-described prohypertensive mechanisms may provide the explanation of a common pathophysiological feature observed in patients with MetS that is sodium sensitivity. Chen *et al.*^[77] evaluated the association between MetS and salt sensitivity of BP in 1906 subjects (with and without MetS). Study participants received a low-sodium diet (3 g sodium chloride per day) for 7 d, followed by a high-sodium diet (18 g sodium chloride per day) for an additional 7 d^[77]. They found that: multi-variable-adjusted mean changes in BP were significantly greater in participants with MetS than in those without on both low-sodium and high-sodium diets^[77].

These results support the notion that patients with MetS, especially those with obesity, are very sensible to sodium intake^[36,45,46].

METABOLIC SYNDROME AND CARDIOVASCULAR RISK

The high prevalence of the MetS is of considerable concern because several studies suggest that people with the MetS are at increased risk for developing T2DM^[18-20,78] and CV events^[18-22,25,27,29-32,78-82].

The ability of MetS to predict the development of T2DM has been examined in numerous studies; it was estimated that the MetS approximately quintuples the risk for incident T2DM^[14,78].

About a hundred longitudinal studies were performed in order to assess the CV prognostic impact of the MetS^[18-22,25,27,29-32,82] and the vast majority of them were included in the four meta-analyses carried out up to now summarizing this issue^[78-81].

The most recent and largest of them was that of Mottillo *et al.*^[81] that included near one million patients (total $n = 951083$). The MetS, defined according to the ATPIII criteria, was associated with a 2-fold increase in risk of CVD, CV mortality, myocardial infarction and stroke, and a 1.5-fold increase in risk of all-cause mortality^[81].

Whether or not the prognostic significance of the MetS exceeds the risk associated with the sum of its individual components is still a matter of debate. Even if a number of studies support the notion that diagnosing the MetS adds nothing beyond each individual risk factor for predicting CVD^[11,12], other investigations^[8,20], such as the METS-GREECE Multicentre study, seem to suggest the opposite^[82]. More recently, in the 19257 hypertensive patients randomized in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm MetS was significantly associated with coronary outcomes, stroke, and all-cause mortality after adjusting for age, sex, and

Table 1 Prospective studies exploring the association of metabolic syndrome with cardiovascular events and all-cause mortality in hypertensive subjects

Ref.	No. of subjects (population)	Mean follow-up (yr)	Mean age (yr)	MetS (%)	MetS definition	T2DM (%)	Risk of all-cause mortality	Risk of CV events
Schillaci <i>et al</i> ^[25]	1742 (Italian hypertensives without CVD at baseline)	4.1	50	34.0	Modified ATP III	6.0	Not reported	HR = 1.73 (1.25-2.38) Cardiac events: HR = 1.48, (1.01-2.27). Cerebrovascular events: HR = 2.11 (1.27-3.50) After exclusion of T2DM HR = 1.43 (1.02-2.08)
Pierdomenico <i>et al</i> ^[27]	802 (Italian hypertensives without T2DM, TOD and CVD at baseline)	6.9	53	27.2	Modified ATP III	0	Not assessed	HR = 2.64 (1.52-4.58)
Andreadis <i>et al</i> ^[29]	1007 (Greek hypertensives without CVD at baseline)	2.1	59	42.1	Modified ATP III	13.2	Not assessed	HR = 1.75 (1.15-2.66) Cardiac events: HR = 1.73 (1.00-3.00). Cerebrovascular events: HR = 1.91 (1.01-3.58) After exclusion of T2DM: HR = 1.67 (1.01-2.74)
Zanchetti <i>et al</i> ^[28]	2034 (European hypertensives participating in the ELSA study)	3.7	56	33.3	Modified ATP III	4.5	Not assessed	Incidence of CV events not different (about 6% in subjects with and in those without MetS)
Pannier <i>et al</i> ^[22]	26447 French hypertensives without CVD at baseline	4.1	50	17.8	ATP III	Not reported	HR = 1.40 (1.13-1.74)	Not assessed
de Simone <i>et al</i> ^[30]	8243 hypertensives with EKG-LVH participating in the LIFE study	4.8	67	19.3	Modified ATP III	12.5	Not assessed	HR = 1.47 (1.27-1.71) CV death: HR = 1.73 (1.38-2.17)
Vlek <i>et al</i> ^[31]	1815 hypertensives with CVD at baseline and without T2DM	3.9	61	42.7	ATP III	0	Not assessed	HR = 1.24 (0.95-1.62) CV death: HR = 1.41 (1.01-1.98)
Gupta <i>et al</i> ^[32]	19257 hypertensives participating in the ASCOT-BPLA study	5.5	63	43.8	ATP III	27.0	HR = 1.35 (1.16-1.58) ¹	Stroke: HR = 1.34 (1.07-1.68) ¹ MI: HR = 1.16 (0.95-1.43) ¹

¹HR are adjusted for the individual components of MetS. MetS: Metabolic syndrome; CVD: Cardiovascular disease; ATP III: Third report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults; T2DM: Type 2 diabetes mellitus; TOD: Target organ damage; CV: Cardiovascular; MI: Myocardial infarction; EKG-LVH: Left ventricular hypertrophy detected by electrocardiography; ELSA: European Lacidipine Study on Atherosclerosis; LIFE: Losartan Intervention For Endpoint reduction; ASCOT-BPLA: Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm.

ethnicity. However, when the model was further adjusted for the individual components, MetS was associated with significantly increased risk of stroke and all-cause mortality but not coronary disease (Table 1)^[32].

The adverse prognostic impact of the MetS, in hypertensive patients was also observed in other six investigations (Table 1)^[22,25,27,29-31]. In the aforementioned PIUMA study, hypertensive participants with MetS had an increased risk of developing cardiac and cerebrovascular events, independently of traditional CV risk factors, including left ventricular (LV) hypertrophy (LVH) and 24-h BP^[25]. Most notably, the association between the MetS and future CV morbidity also held in patients without diabetes mellitus at the baseline examination^[25].

In contrast with these studies, in the European Laci-

dipine Study on Atherosclerosis study, a large cohort of well-treated hypertensive subjects, outcomes were not different between patients with MetS and those without it^[28], probably because an effective antihypertensive treatment may largely counteract the detrimental influence of MetS (Table 1).

It is conceivable that the increased CV risk conferred by MetS in hypertensive subjects may in part be mediated through preclinical cardiac and renal organ damage. Indeed, major CV events in most hypertensive patients are preceded by the development of asymptomatic cardiovascular and renal structural and functional abnormalities^[83], most of which are recognized as significant independent predictors of adverse cardiovascular outcomes^[84-87].

Table 2 Cross-sectional studies investigating the association of metabolic syndrome with various markers of subclinical organ damage

Ref.	No. of subjects (population)	LVM	LV diastolic function	Carotid IMT and plaques	Micro- albuminuria	CKD	Arterial stiffness
Mancia <i>et al</i> ^[21]	2051 (Italian GP)	↑	-	-	-	-	-
Cuspidi <i>et al</i> ^[23]	447 (Italian hypertensives)	↑	-	↑	↑	-	-
Leoncini <i>et al</i> ^[24]	354 (Italian hypertensives)	↑	-	↑	↑	-	-
Mulè <i>et al</i> ^[26]	353 (Italian hypertensives)	↑	Impaired	-	↑	-	-
Mulè <i>et al</i> ^[91]	475 (Italian hypertensives)	↑	Impaired	-	-	-	-
Schillaci <i>et al</i> ^[92]	618 (Italian hypertensives)	↑ ¹	Impaired ¹	-	-	-	-
Nicolini <i>et al</i> ^[93]	200 (Italian hypertensives)	↑ ¹	Impaired ¹	-	-	-	-
Aijaz <i>et al</i> ^[94]	2042 (United States GP)	↑ ¹	Impaired ¹	-	-	-	-
Sundström <i>et al</i> ^[96]	820 (elderly Swedish GP)	↑	-	-	-	-	-
de Simone <i>et al</i> ^[97]	2758 (American Indian GP)	↑	Impaired	-	-	-	-
Burchfiel <i>et al</i> ^[98]	1572 (United States Black GP)	↑	-	-	-	-	-
de las Fuentes <i>et al</i> ^[99]	607 (United States GP)	↑	Impaired	-	-	-	-
Hwang <i>et al</i> ^[100]	1599 (South Korean GP)	↑	Impaired	-	-	-	-
Kim <i>et al</i> ^[101]	1886 (South Korean GP)	-	Impaired	=	-	-	↑
Ingelsson <i>et al</i> ^[102]	1945 (United States GP)	↑	-	↑	↑	-	-
Ferrara <i>et al</i> ^[103]	340 (Italian hypertensives)	↑	-	=	-	-	-
Aksoy <i>et al</i> ^[105]	90 (Turkish subjects)	↑	Impaired	-	-	-	-
Mulè <i>et al</i> ^[88]	93 (Italian hypertensives)	-	-	-	-	-	↑
Schillaci <i>et al</i> ^[119]	169 (Italian hypertensives)	-	-	-	↑	-	↑
Scuteri <i>et al</i> ^[120]	20750 (9 cohorts from Europe and United States)	-	-	-	-	-	↑
Scuteri <i>et al</i> ^[121]	6148 (Italian GP aged 14-102 years)	-	-	↑	-	-	↑
Scuteri <i>et al</i> ^[122]	471 (United States GP)	-	-	↑	-	-	↑
Zanchetti <i>et al</i> ^[128]	2034 (European hypertensives)	-	-	↑	-	-	-
Kawamoto <i>et al</i> ^[124]	760 (Japanese patients)	-	-	↑	-	-	-
Irace <i>et al</i> ^[125]	1853 (Italian GP)	-	-	=	-	-	-
Chen <i>et al</i> ^[110]	6217 (United States GP)	-	-	-	↑	↑	-
Chen <i>et al</i> ^[109]	15160 (Chinese GP)	-	-	-	↑	↑	-
Navarro <i>et al</i> ^[111]	8425 (Spanish hypertensives)	-	-	-	-	↑	-
Johns <i>et al</i> ^[112]	574 (United States non-diabetic GP)	-	-	-	-	↑	-

¹Only in women. LVM: Left ventricular mass; IMT: Intima-media thickness; CKD: Chronic kidney disease; =: No difference; ↑: Increased; -: Not evaluated; LV: Left ventricular; GP: General population.

METABOLIC SYNDROME AND HYPERTENSIVE TARGET ORGAN DAMAGE

The very frequent occurrence of BP values in the high normal or frankly hypertension range in subjects with the MetS^[18-21] may explain the increased prevalence of hypertension-related preclinical (or asymptomatic) organ damage, such as LVH, elevated urinary albumin excretion rate and arterial stiffening^[18-21,23,24,26]. Some of these markers of organ damage, however, are frequently observed also in individuals who have the MetS without a BP elevation, or also in hypertensive individuals after adjustment for BP values in multivariate analyses, suggesting that other components of this condition play a role independently of BP^[20] (Table 2).

We performed a cross-sectional study to assess the impact of MetS, defined according to the NCEP-ATP III criteria, on some cardiac, renal and retinal markers of target organ damage (TOD), in 353 non-diabetic young and middle aged essential hypertensives without clinical or laboratory evidence of CV and renal diseases^[26].

In a subset of untreated subjects of the same population, we also explored the carotid-femoral pulse wave velocity (PWV), a measure of aortic stiffness, in patients with and without MetS^[88].

Hypertensive patients with MetS exhibited higher LV mass on echocardiography [either normalized by body surface area (BSA) or by height elevated by a power of 2.7], relative wall thickness, left atrial size, and greater prevalence of LV hypertrophy, lower mid-wall fractional shortening and a longer E-wave deceleration time than subjects without MetS^[26]. These results were maintained even after correction for several confounding variables, such as age, gender distribution, severity and duration of hypertension and previous antihypertensive therapy. In particular, after adjustment for these covariates, the likelihood of LV hypertrophy was 2.89-fold (95% interval, 1.68 to 4.98) higher in subjects with MetS than in those without it, when LV mass was indexed by height^{2.7} (LVMH^{2.7})^[26]. Moreover, the higher the number of components of the MetS, the greater the LVMH^{2.7}^[18]. It is noteworthy that the relationship between MetS and LV mass was confirmed in multivariate regression models, including MetS together with its individual components, as independent variables^[26]; this seems to suggest that MetS may have a deleterious effect on cardiac structure over and above the potential contribution of each single component of this syndrome, and that the confluence of abnormalities that comprise MetS may have a synergistic negative impact on LV mass.

We obtained similar results also when the influence of MetS on cardiac mass was evaluated in white coat hyper-

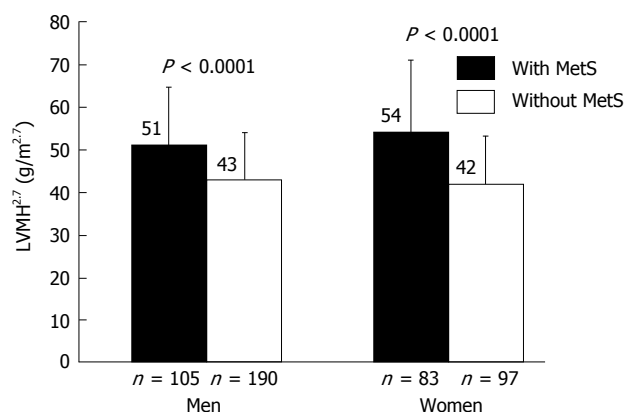


Figure 2 Mean values of left ventricular mass indexed for height^{2.7} in hypertensive men and women with and without the metabolic syndrome^[91]. LVMH^{2.7}: Left ventricular mass indexed for height^{2.7}; MetS: Metabolic syndrome.

tensives^[89], and in a subgroup of overweight and obese hypertensive patients^[90]. On the other hand we did not observe a significant effect modifier of gender on the association between MetS and LV mass^[91], at variance with the results reported in some^[92-94], but not all^[95,96], investigations exploring this issue.

Indeed, we found similar differences, regarding LV mass, in females and males with MetS when compared to their counterparts without the MetS (Figure 2)^[91]. Moreover, in a two-factor ANOVA model, the analysis of the interaction term “gender × MetS” revealed no significant effect of sex on the association between MetS and LV mass, either normalized for BSA or height^{2.7}^[91].

The unfavorable impact of the MetS on cardiac structure was confirmed in a large number of cross-sectional studies, conducted in different ethnic groups, in general populations^[97-102], as well as in hypertensive patients^[18-20,103] (Table 2). Moreover, it was even more convincingly demonstrated by the population based PAMELA study, in which the subjects with MetS had a three fold risk to develop LVH, than those without it, during a ten years follow-up period^[104].

The putative mechanisms by which MetS promotes LVH^[18,19] are summarized in Figure 3. It is interesting to note that a variety of studies suggest that LV diastolic function may be adversely influenced by the MetS *per se*^[99-101], even in the absence of diabetes and hypertension and in part independently of age and left ventricular mass^[105].

The asymptomatic changes in cardiac structure and function induced by the MetS largely explain why this syndrome is a powerful independent predictor of subsequent heart failure (HF), even after adjustment for established risk factors for HF^[106,107]. This increased HF risk may be in part promoted by insulin resistance and accompanying hyperinsulinemia that may have direct myocardial effects in addition to its proatherosclerotic effects. Indeed, in the Uppsala Longitudinal Study of Adult Men, insulin resistance, measured with the reference standard euglycaemic insulin clamp technique, was an independent

risk factor for HF, taking diabetes, obesity and other potential confounding factors into account^[108].

There are other important findings from our study that deserve special mention: hypertensive subjects with MetS compared to those without it showed greater level of albumin excretion rate and consequently higher prevalence of microalbuminuria^[26], that is nowadays considered, not only a predictor of renal complications, but also a harbinger of premature CVD^[86,87]. These results, that we confirmed in the larger population of the above described REDHY study^[35] (Figure 4), are consistent with the findings of other investigations conducted in hypertensive patients^[23,24] and in general populations^[102,109]. In some of these studies^[109] and in other ones^[110-113], with cross-sectional and longitudinal design, a relationship between the MetS and chronic kidney disease was also observed (Table 2).

Another result of our study merits a comment. In keeping with other reports^[114,115], we noted an increased prevalence of grade I and grade II hypertensive retinopathy in subjects with MetS when compared to persons without MetS^[26]. However, because the prognostic implications of early hypertensive retinopathy grades are unclear^[116], the clinical significance of these findings remains undefined.

Unlike the milder forms of hypertensive retinopathy, prognostic value of increased aortic stiffness seems to be more soundly demonstrated; there is an extensive and very consistent body of evidence showing that large artery stiffening is a powerful predictor of CV morbidity and mortality^[85]. Because a fundamental principle states that pulse waves travel faster in stiffer arteries, PWV is the most widely used measure of arterial stiffness. PWV measured along the aortic and aorto-iliac pathway is the most clinically relevant since the aorta and its first branches are responsible for most of the pathophysiological effects of arterial stiffness^[85]. Therefore, aortic PWV is regarded as the gold standard measurement of arterial stiffness.

When we assess the influence of MetS on aortic PWV in a sample of never treated non-diabetic patients with essential hypertension, we found more elevated PWV in subjects with MetS when compared to those without it^[88].

These data, that we recently replicated in a wider group of hypertensive patients (Figure 5), are in line with the results we observed in another cross sectional study carried out in 528 nondiabetic patients (age 18 to 72 years) with essential hypertension^[116]. We found that, when compared with subjects without MetS, hypertensive patients with MetS exhibited more elevated clinic and 24-h pulse pressures that may be considered as a proxy for arterial stiffness, especially in older subjects. The difference held even after correction for age, sex, stroke volume, mean pressures, and total cholesterol^[116]. The regression line relating PP with age was steeper in patients with MetS than in those without MetS (Figure 6), suggesting that arterial aging is faster in the former as compared to the latter^[117].

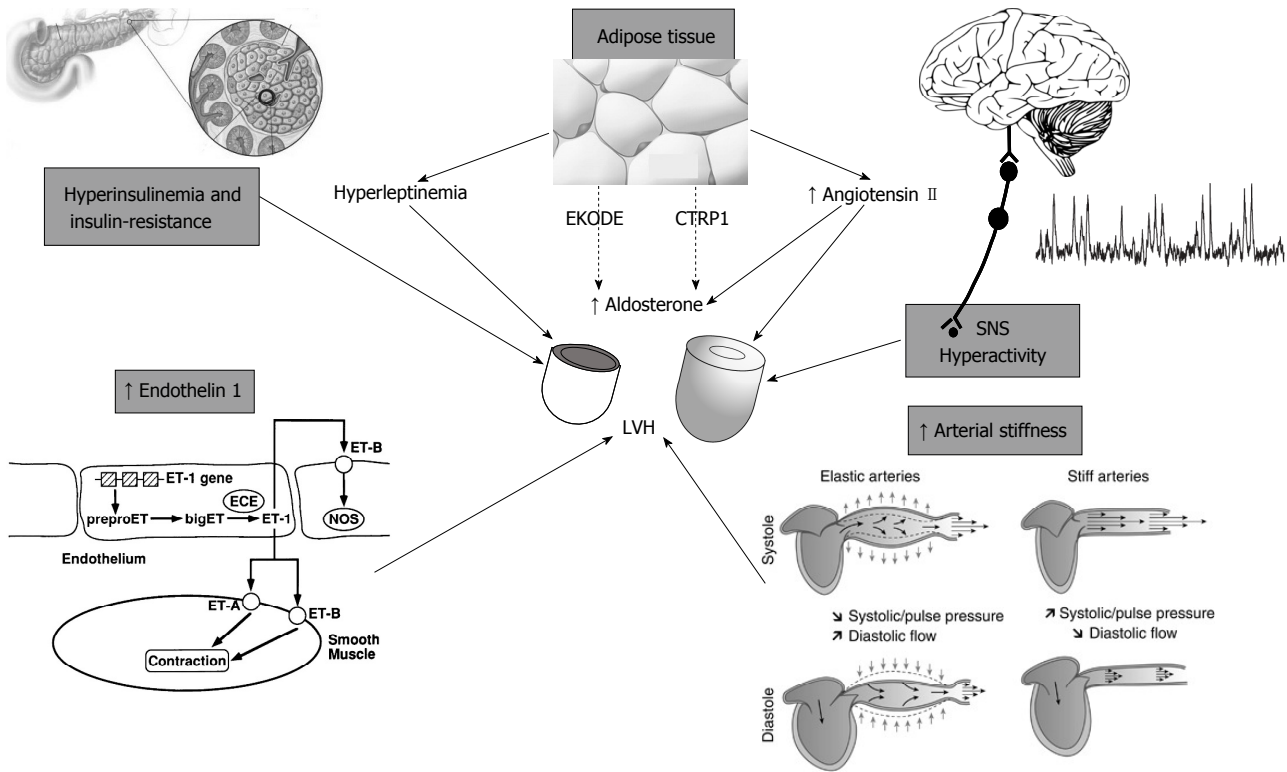


Figure 3 Putative mechanisms by which metabolic syndrome promotes left ventricular hypertrophy. SNS: Sympathetic nervous system; CTRP1: Complement-C1q Tumor necrosis factor-related protein 1; EKOGE: Epoxy-keto derivative of linoleic acid; LVH: Left ventricular hypertrophy.

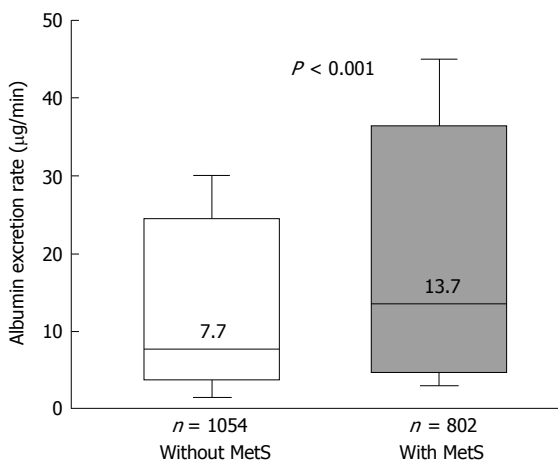


Figure 4 Box plots showing urinary albumin excretion rates in nondiabetic hypertensives participating in the Renal Dysfunction in Hypertension study^[39], divided in subjects with and without the metabolic syndrome. In the Box-and-Whisker plots, the central boxes represent the interquartile range (25th to 75th percentile). The middle lines, and the numbers above these lines, represent the median values. Lower and upper whiskers extend to 5th and 95th percentile. MetS: Metabolic syndrome.

Our observations are in agreement with several lines of evidence^[101,118-122], suggesting that the MetS accelerates the age-related rise in arterial stiffness, leading to a condition defined as early vascular aging (EVA)^[123]. Premature arterial senescence in MetS is biologically plausible. The structural changes occurring during aging in large arteries include extensive impairment of the elastin fiber network, increase in collagen content, calcification of the

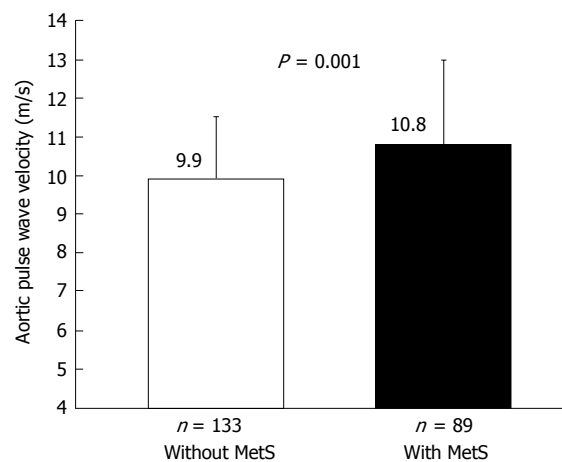


Figure 5 Mean values of aortic pulse wave velocity in untreated hypertensive subjects with and without the metabolic syndrome^[76]. MetS: Metabolic syndrome.

media, and accumulation and migration of VSMC in the arterial walls^[121,123]. In subjects with MetS, these modifications may occur earlier, especially in the aorta, for several reasons: (1) activation of the RAAS, that is involved in regulating the turnover of extracellular matrix proteins and that is a strong regulator of matrix metalloproteinase and tissue inhibitor of metalloproteinases; (2) increase in oxidative stress and chronic low grade inflammation; (3) increased glycation of matrix proteins; (4) decreased endothelial bioavailability of nitric oxide associated with insulin resistance; (5) endothelin-1 increase; (6) elevation

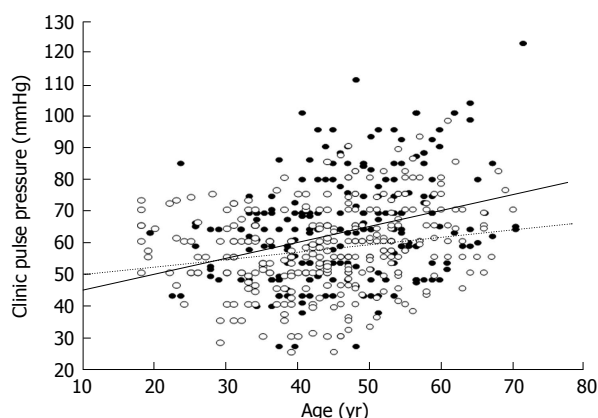


Figure 6 Scatterplot showing the relationship between age and pulse pressure in subjects with (black circles) and in those without (white circles) metabolic syndrome. The calculated regression lines for the former (continuous line) and the latter patients (dotted line) were also shown. The difference regarding the slopes of the two regression lines was statistically significant ($P = 0.01$).

in leptin; and (7) hypoadiponectinemia^[18-20].

EVA, as well as other indices of preclinical organ damage, reflects cumulative damaging effects from risk factors and entails an enhanced risk of CV events and of cognitive dysfunction^[123]. Schillaci *et al.*^[119] in 169 newly diagnosed non-diabetic hypertensive subjects, observed a greater aortic PWV in the subgroup with MetS, whereas upper limb PWV did not differ in the groups with and without MetS. Very recently, Scuteri *et al.*^[120] studied 20570 subjects from 9 cohorts representing 8 different European countries and the United States, participating in the Metabolic syndrome and Arteries REsearch (MARE) Consortium. In this large-scale observational study any cluster of MetS components identified as MetS, with the exception of low HDLc (H) + high triglycerides (T) + abdominal obesity (W), was associated with stiffer arteries than in control subjects^[120]. Overall, the combinations T + elevated BP (B) + W, elevated fasting glucose (G) + B + W, and G + T + B + W were consistently associated with significantly stiffer arteries to an extent similar or greater than observed in subjects with alteration in all the five MetS components, even after adjustment for multiple confounders. Differences in BP levels amongst the clusters of MetS components do not seem to explain the reported difference in the odds of having stiffer arteries^[120]. The results attained in the MARE Consortium concur with those obtained in the SardiNIA Project^[121] and in the Baltimore Longitudinal Study on Aging^[122] where the subject with MetS showed an increased carotid stiffness when compared with subjects without it.

Moreover, the results of these studies support the notion that the MetS accelerates arterial ageing over and above the predicted power of its individual components, in marked contrast with the concept that the MetS does not provide further information in addition to the sum of its components. In the same investigations an association between MetS and carotid intima-media thickness has been observed^[121,122], in accordance with some^[23,24,28,102,124],

but not all studies^[101,125].

A significant association between carotid atherosclerosis and MetS has been reported in participants in the Framingham Offspring study with MetS^[102]. In the same study a greater prevalence of various indices of subclinical CVD (left ventricular hypertrophy by electrocardiography or echocardiography, carotid ultrasound abnormalities, reduced ankle-brachial index, microalbuminuria) was described in subjects with MetS^[102].

Interestingly, individuals with MetS with evidence of subclinical disease experienced a risk of CV events nearly threefold that of participants without subclinical disease. The presence of subclinical disease conferred approximately a two-fold risk of overt CVD even in those without either MetS or diabetes (compared with their counterparts without subclinical disease). Adjustment for subclinical disease presence markedly attenuated the association of MetS with CVD risk^[102]. This observation emphasizes the important role of subclinical disease in mediating the CV risks associated with MetS.

METABOLIC SYNDROME AND HYPERTENSION: THERAPEUTIC IMPLICATIONS

Effective CVD prevention requires that multiple risk factors be addressed simultaneously to obtain the most significant reduction of morbidity and mortality in a given population. From this point of view, the identification of patients with the MetS offers a unique chance of practicing preventive medicine.

Once identified, aggressive treatment of the MetS is crucial to reduce the increased CV risk. Medications are targeted to individual components of the syndrome (Table 3). However, although pharmacological therapy is often necessary, the cornerstone of treating the MetS remains lifestyle modification^[5,20], that represents the only truly holistic therapeutic approach that can reduce insulin resistance and visceral obesity. It involves behavioral counseling, education, dietary changes and increased physical activity, with a goal of ≥ 30 min of moderate-intensity activity on most days of the week^[5,20]. Even modest weight loss (7% to 10% of body weight) results in decreased fat mass, BP, glucose, and triglyceride levels^[5,20]. These benefits can also translate into improved long-term outcome, especially if weight loss and lifestyle alterations are maintained.

A meta-analysis of 50 studies and 534906 individuals showed that adherence to the Mediterranean diet protect against the development of the MetS and its individual components. This dietary pattern, that can be easily followed by various cultures with small modifications, is characterized by the frequent consumption of olive oil, fruits, tree nuts, legumes, whole grains, weekly consumption of fish and poultry, a relatively low consumption of red meat, as well as a moderate consumption of alcohol normally with meal and usually in the form of

Table 3 Therapeutic approaches in patients with metabolic syndrome

Metabolic syndrome component	Goal of therapy	Drugs	Diet	Physical exercise
Arterial hypertension	BP < 140/90 mmHg	ACEI or ARBs and/ or Ca-antagonists and/ or alpha-blockers ¹ Limit diuretics and beta-blockers	Salt restriction and hypocaloric	Regular exercise
Hyperglycemia	HbA1c < 7%-6.5%	Metformin GLP-1-Agonists DPP-4-inhibitors	Hypocaloric	Regular exercise
Obesity	Weight loss 7%-10%	Orlistat Bariatric Surgery	Hypocaloric	Regular exercise
Dyslipidemia	LDL < 100-70 mg/dL TG < 150 mg/dL HDL: Men > 40/ Women > 50 mg/dL	Statins ± ezetimibe. PUFA-n-3, Fibrates	Hypocaloric	Regular exercise

¹Not first choice. BP: Blood pressure; ACEI: Angiotensin converting enzyme inhibitors; ARBs: Angiotensin II receptor blockers; GLP-1: Glucagon-like peptide-1; DPP-4: Dipeptidyl peptidase-4; LDL: Low-density lipoprotein; TG: Triglycerides; HDL: High-density lipoprotein; PUFA-n-3: Omega-3-Polyunsaturated.

red wine^[126]. However, the remaining challenge is how to promote long-term adherence to a healthier, more active lifestyle and avoid reversion to old habits.

The more recent European guidelines for the management of hypertension do not recommend prescribing antihypertensive drugs in subjects with high normal BP, because no evidence is available^[127]. The same guidelines do point out that beta-blockers (except for vasodilating beta-blockers) and diuretics (especially when combined together) may facilitate the development of new onset diabetes and therefore should be avoided as first line therapy in hypertensives with MetS^[127]. When diuretics are employed, low doses should be used, preferably in association with a potassium-sparing drug, because hypokalemia may worsen glucose metabolism^[127].

Unlike beta-blockers and diuretics, newer antihypertensive medications are associated with a reduced (or not increased) risk of incident diabetes^[20,51,127] and they are also associated with better adherence to therapy^[128]. In addition, it has been demonstrated that obese hypertensive patients during weight loss therapy show significantly better weight reduction and improvement of insulin resistance when treated with newer antihypertensive medications compared to the older BP lowering drugs (especially beta-blockers)^[20,65,127]. Of the newer antihypertensive treatments angiotensin receptor blockers (ARBs) have been found to be associated with lowest rate of discontinuation of therapy^[128] and with lowest incidence of new onset diabetes^[129]. Moreover, specific ARBs (telmisartan and to a lesser extent irbesartan) seem to allow a superior control of BP over 24 h, documented also in subjects with MetS^[130] and also a partial peroxisome proliferator-activated receptor- γ agonism not present in other ARBs or ACE-inhibitors (ACEI)^[20,127,131]. However, the clinical relevance of these differences seem to be negligible or uncertain, since in the Ongoing Telmisartan Alone and in Combination with Ramipril Trial, telmisartan was not more effective than ramipril in preventing CV events or delaying onset of diabetes^[127].

The choice of the newer BP lowering drugs, such as the RAAS-blockers and the long-acting calcium antagonists, seems to be particularly recommended in hypertensive patients with MetS, in the light of the above-mentioned marked tendency of these subjects to the development of LVH and stiffening of the large arteries^[18-26]. As a matter of fact, the efficacy of these drugs, in reducing LV mass and arterial stiffness^[127] is greater than the older ones.

Although meta-analyses suggest antihypertensive drugs have a similar effect on reducing CV events^[127], no randomized clinical trial has been specifically performed in hypertensive patients with the MetS, having the aim to test the superiority of one class of BP lowering drugs over another. However, very recently, in the Cardiovascular Health Study, a community-based prospective cohort study conducted by the National Heart Lung and Blood Institute, the association between the use of ACEI/ARBs and incident CV events was evaluated in elderly people with hypertension and MetS^[132]. ACEI/ARBs use was associated with a lower risk of CVD events, primarily due to a reduction in coronary events^[132]. Pending validation from prospective clinical trials, it seems reasonable to say that ACEI/ARBs may be the preferred treatment for hypertension management in patients with MetS.

Newer antihypertensive agents lead to better control of BP in part brought about by better adherence, thereby reducing the risk of CVD. Needless to say that CV events and new onset T2DM are associated with significant social and health costs. Therefore, in patients with hypertension and MetS, some of the drug costs of newer antihypertensive medications will be balanced by costs saved from reducing these negative outcomes.

CONCLUSION

An extensive body of evidence suggests that the MetS may accelerate arterial aging and amplify hypertension-related cardiac and renal changes. Some of the MetS

components, when considered individually may have little or no influence on TOD, but when taken together may synergistically interact promoting the development of LV hypertrophy, LV diastolic dysfunction, aortic stiffness and microalbuminuria. The marked tendency of the hypertensive patients with the MetS to develop these preclinical manifestations of end-organ damage, may largely explain why the MetS entails an increased risk of CV morbidity and mortality, since these markers of TOD are well-known predictors of CV events. Therefore, identifying the MetS in hypertensive patients may enable the clinician to better assess the CV risk. Once this syndrome is properly identified, aggressive implementation of therapeutic lifestyle changes and appropriate medications, able to decrease insulin resistance, hyperinsulinemia and weight gain can greatly reduce its adverse prognostic impact.

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WJC 6th Anniversary Special Issues (1): Hypertension

Management of erectile dysfunction in hypertension: Tips and tricks

Margus Viigimaa, Charalambos Vlachopoulos, Antonios Lazaridis, Michael Doumas

Margus Viigimaa, Tallinn University of Technology, Institute of Biomedical Engineering, Tallinn 13419, Estonia

Margus Viigimaa, North Estonia Medical Centre, Tallinn 13419, Estonia

Charalambos Vlachopoulos, 1st Department of Cardiology, Kapodestrian University, Athens 11527, Greece

Antonios Lazaridis, Michael Doumas, 2nd Propedeutic Department of Internal Medicine, Aristotle University, Thessaloniki 54642, Greece

Author contributions: All authors contributed equally to the preparation of the manuscript.

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Correspondence to: Margus Viigimaa, Professor, North Estonia Medical Centre, Sütiste St. 19, Tallinn 13419, Estonia. margus.viigimaa@regionaalhaigla.ee

Telephone: +372-61-71415 Fax: +372-61-71415

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Core tip: The prevalence of erectile dysfunction is approximately 2-fold higher in hypertensive patients compared to normotensive individuals. However, erectile dysfunction remains under-reported, under-recognized, and under-treated in hypertensive patients. Lifestyle modification should be the mainstay of treating erectile dysfunction in patients with untreated hypertension. Switching antihypertensive therapy should be considered in treated hypertensive patients, unless administered drugs are absolutely indicated for the individual patient. Otherwise, phosphodiesterase-5 inhibitors should be used, since they are both effective and safe in hypertensive patients. Finally, erectile dysfunction offers the opportunity to recognize asymptomatic cardiovascular disease with obvious benefits for cardiovascular event prevention.

Abstract

Arterial hypertension is a major risk factor for cardiovascular disease and affects approximately one third of the adult population worldwide. The vascular origin of erectile dysfunction is now widely accepted in the vast majority of cases. Erectile dysfunction is frequently encountered in patients with arterial hypertension and greatly affects their quality of life of hypertensive patients and their sexual partners. Therefore, the management of erectile dysfunction in hypertensive patients is of paramount importance. Unfortunately, erectile dysfunction remains under-reported, under-recognized, and under-treated in hypertensive patients, mainly due to the lack of familiarity with this clinical entity by treating physicians. This review aims to discuss the more frequent problems in the management of hypertensive patients with erectile dysfunction and propose ways to overcome these problems in everyday clinical practice.

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INTRODUCTION

Undoubtedly, heart disease is and will continue to be one of the major health problems of modern society. Approximately one death every forty seconds occurs due to cardiovascular (CV) disease in the United States alone and arterial hypertension is one of the greatest culprits for it^[1]. Considering the fact that around 25% of the global

population suffer from arterial hypertension, predicted to reach 1.5 billion people in the foreseeable future, it is easily deducted that a respectful part of the general population is under major and constant CV risk^[2,3].

In addition, those patients experience a lower health quality and exhibit lower scores in the widely acceptable quality of life parameters. Sexual dysfunction, an acknowledged condition frequently co-existing with hypertension, contributes significantly to the impaired health quality of both hypertensive patients and their sexual partners^[4,5].

An equally valuable observation though, is the fact that sexual dysfunction could indeed indicate asymptomatic CV disease. A solid amount of evidence accumulated over the last years has pointed out towards that trend moving, hesitatingly though, sexual dysfunction in the surface of scientific interest. As such, commonly under-reported, under-recognized and under-treated, sexual dysfunction could indeed play its role in cardiovascular risk assessment and stratification.

Despite physician's inexperience and patient's reluctance to disclose sexual dysfunction problems, attempts to estimate the magnitude of this clinical condition have predicted that over 150 million men worldwide experience some degree of erectile dysfunction. Several studies have demonstrated a wide range regarding the prevalence of erectile dysfunction, which is even higher in patients with essential hypertension where the relative risk is approximately two times higher than in normotensive individuals^[6-11]. The etiology can be found in the structural and functional abnormalities of the penile arteries induced by high blood pressure. Smooth muscle hypertrophy, stenotic lesions due to atherosclerosis and impaired blood flow are among the prominent structural alterations whereas endothelial dysfunction and the defective nitric oxide-induced vasodilatory mechanism belong to the main functional abnormalities induced by increased blood pressure^[12,13]. As a matter of fact, sexual dysfunction is encountered more frequently that it is indeed believed underlining the need for a more proper and concrete assessment.

This review aims to discuss the more frequent problems in the management of hypertensive patients with erectile dysfunction and propose ways to overcome these problems in everyday clinical practice.

UNTREATED HYPERTENSIVE PATIENTS

Vasculogenic sexual dysfunction is the main cause of sexual dysfunction in untreated hypertensive patients. However, due to the complex etiologic and pathophysiologic nature of sexual dysfunction, exclusion of concomitant diseases and drugs should be the initial step when approaching a hypertensive patient with this clinical condition that is not receiving any antihypertensive medication. Consequently, a significant amount of neurological, psychiatric, urologic and endocrine disorders should be ruled out before vasculogenic sexual dysfunction is diagnosed.

When the diagnosis of vasculogenic sexual dysfunction

has been carefully reached, physicians will have to come up with an effective treatment. Appropriate lifestyle measures and adoption of a healthier attitude could represent an initial, efficient and cost-effective treatment option^[14]. This is due to the fact that traditional CV risk factors such as hypertension, physical inactivity-obesity, smoking and dyslipidemia have been consistently linked with endothelial and consequently sexual dysfunction^[15]. In this context, it has been demonstrated that moderate physical activity can reduce up to 30% the risk of erectile dysfunction contrary to sedentary life, which exerts a deleterious effect^[16]. Interestingly, the beneficial effect of physical exercise on sexual dysfunction seems to be independent of its favorable impact on the general cardiovascular profile^[17]. In terms of caloric reduction, Mediterranean diet exerts a positive effect on sexual function parameters of patients with metabolic syndrome^[18]. Moreover, combined physical exercise and caloric restriction can result in weight reduction which in succession can reduce up to 30% the risk of obesity-associated erectile dysfunction^[19].

Whereas lifestyle modification is a reasonable initial step when approaching a hypertensive patient with sexual dysfunction, finding the appropriate antihypertensive treatment is usually the next "complicated" move to care for. Several observational and clinical studies have consistently associated antihypertensive medication with sexual dysfunction^[20]. Whether one class of antihypertensive agents is associated exclusively or more with erectile dysfunction compared to another, however, is a difficult puzzle to solve as there are many other factors (comorbid conditions, concomitant medications, personal characteristics) to be taken into account at the same time. In addition, erectile dysfunction has never been studied as the primary end-point before and as a result a definite causative relationship between antihypertensive medication and sexual dysfunction has never been proven.

Despite the existing controversies, available data so far imply the old generation β -blockers (*e.g.*, propranolol) as the major culprits for sexual dysfunction with the newer ones (carvedilol, celiprolol) to exert a less pronounced negative effect^[21-24]. A luminous exception to the rule, nebivolol, is a newer agent of its class which significantly ameliorates erectile dysfunction through increased nitric oxide generation, an effect consistently demonstrated in recent studies^[25,26]. Diuretics, even on adjunct therapy, constitute another antihypertensive agent negatively associated with sexual function^[27-29]. On the other hand, calcium antagonists and angiotensin converting enzyme inhibitors seem to demonstrate a neutral effect^[30-32]. Interestingly, angiotensin receptor blockers (ARBs) by blocking the vasoconstrictive action of angiotensin II seem to positively affect erectile function and are thus regarded as a first-line treatment in hypertensive patients with erectile dysfunction^[22,25,33-35].

TREATED HYPERTENSIVE PATIENTS

Whereas management of sexual dysfunction in previ-

ously untreated hypertensive patients can be a challenging procedure, confronting the same clinical condition in individuals under antihypertensive regime can be even more demanding. In such cases there will always be a question hovering over physicians head. Is hypertension *per se*, antihypertensive medication or both, the causative factors provoking sexual dysfunction^[15]?

Duration and severity of hypertension are undoubtedly associated with erectile dysfunction. As a result, patients with long-standing (> 5-6 years) and severe hypertension are expected to suffer more frequently from sexual dysfunction, which indeed appears in a more severe form^[36,37].

When antihypertensive medication comes to the fore, certain issues need to be carefully addressed. This is due to the fact that medically induced erectile dysfunction is one of the major reasons for non-adherence and treatment discontinuation, a reality that could have deleterious consequences on patient's cardiovascular profile and health quality in the long term^[38,39].

Like the case of untreated hypertensive patients, evaluation of sexual dysfunction in hypertensive patients under antihypertensive regime, should primarily exclude other concomitant diseases and pharmaceutical agents. Consecutively, a competent physician with advanced communicational skills should try to "discover" medically induced erectile dysfunction since a vast majority of patients being under complex antihypertensive regimes usually attribute the undesirable effect to normal aging thus not relating it to their current medication. Moreover, even physicians seldom report the cases of sexual dysfunction associated with certain medications. When medically induced sexual dysfunction is finally disclosed and a shift in medication is deemed necessary, b-blockers along with diuretics should generally be the first categories to be changed, unless they are deemed absolutely indicated for the individual patient. Ideally, an ARB could constitute the mainstay of therapy in these cases. If sexual dysfunction still persists, then more effective remedies should be elected paving the way for the introduction of phosphodiesterase-5 inhibitors (PDE-5).

PDE-5 inhibitors

Since their introduction in the therapeutic field, more than a decade ago, PDE-5 inhibitors have revolutionized the treatment of sexual dysfunction. By blocking the activity of PDE-5 isoenzyme, localized throughout the smooth muscle cells of the vasculature (genital vessels included), PDE-5 inhibitors increase the levels of cyclic guanosine monophosphate thus exerting vasodilating properties and facilitating penile erection^[40-42]. Due to these properties, sildenafil was the first drug of its class to receive wide acceptance. Its short half-life, food interactions and the associated visual disturbances however, paved the way for the development of newer PDE-5 inhibitors. As such vardenafil with its more rapid onset of action, and tadalafil with its longer half-life and the lack of food interactions or side effects, have offered signifi-

cant alternatives to sildenafil^[43-50].

Due to their vasorelaxing effect, administration of PDE-5 inhibitors in hypertensive individuals was initially confronted with great suspicion. A wealth of clinical data however has proven that PDE-5 inhibitors are associated with few side effects and provoke a small and insignificant reduction in blood pressure with minimal heart rate alterations in both normotensive and hypertensive patients as well. As a matter of fact, they can be safely and effectively administered to hypertensive individuals even when they are already taking multiple antihypertensive agents^[51-56]. The sole exception to the rule is co-administration with organic nitrates, which is an absolute contraindication due to profound and possibly hazardous hypotension effect^[57,58]. Moreover, precaution should be taken when PDE-5 inhibitors are combined with a-blockers where, due to possible orthostatic hypotension effect, lower starting doses should be implemented in the therapeutic regime^[59-62].

Apart from their beneficial effect in erectile dysfunction and their safe profile in antihypertensive medication, PDE-5 inhibitors have even more advantages to demonstrate. Several lines of evidence has proven that patients receiving PDE-5 inhibitors are more likely to initiate an antihypertensive regime and more willing to add a new agent to their existing treatment, a fact that raises significantly patient's adherence and as a matter of fact control of high blood pressure and quality of life^[63,64]. Moreover, a handful of clinical data has demonstrated the considerable vasodilating and anti-proliferative properties of PDE-5 inhibitors in the pulmonary vasculature, establishing them as a first-line treatment in patients with pulmonary arterial hypertension^[65,66]. The same properties have been considered as potentially responsible for improving microcirculation in patients with secondary Raynaud phenomenon and ameliorating cardiopulmonary exercise performance in patients with heart failure^[67,68]. In addition, the therapeutic implementation of PDE-5 inhibitors has expanded in the field of benign prostate hyperplasia-lower urinary tract symptoms (BPH-LUTS). The common pathophysiologic substrate between erectile dysfunction and BPH-LUTS has rendered PDE-5 inhibitors an effective treatment which significantly improves measures of both conditions while at the same time exhibits high efficacy and safety. The beneficial effect is much more pronounced when taking into consideration the fact that a-blockers, the mainstay of therapy for benign prostate hyperplasia frequently provoke sexual side effects, erectile dysfunction included^[69].

Management beyond PDE-5 inhibitors

Despite remarkable therapeutic efforts, it is evident that a relative proportion of patients with erectile dysfunction will fail to respond to oral pharmacotherapy including PDE-5 inhibitors. The management of non-responders calls for second and third-line treatment implementation.

Surgical implantation of a penile prosthesis, either the inflatable (2- and 3-piece) or the malleable device, is a

feasible technique that offers a third-line treatment and a more permanent solution to the problem of erectile dysfunction. Interestingly, prosthesis implantation receives a significantly high satisfaction rate as evidenced by the proportionate scores in sexual satisfaction scales. Mechanical failure and infection are the two major disadvantages of those prosthetic implants however, their great efficacy, safety and satisfaction rate in general render them an attractive solution when conservative treatment fails^[70-74].

CARDIOVASCULAR RISK PREDICTION

One of the most interesting aspects considering the properties of sexual dysfunction is that, during the last decades, it transformed from being a reliable quality of life index into a significant CV risk predictor.

Towards this direction, several sufficiently powered studies have demonstrated a higher incidence of erectile dysfunction in patients with coronary artery disease, either asymptomatic or overt. At the same time, patients with erectile dysfunction are more prone to have established coronary artery stenosis of more than 50% and consequently evident CV disease^[75]. This is in conformity with the “artery size hypothesis” according to which smaller arteries (*e.g.*, penile arteries) are the first to undergo a vascular lesion prior to the larger ones (*e.g.*, coronary arteries). Moreover, in such patients erectile dysfunction is connected to the number of occluded vessels and more interestingly occurs over three years before coronary artery disease becomes apparent^[76-80].

Several other facts support the close relationship between sexual dysfunction and CV disease. Endothelial dysfunction mediated by decreased nitric-oxide bioavailability as well as atherosclerotic lesions constitute a common pathophysiologic substrate affecting both CV disease and erectile dysfunction, a disease considered to be primarily of vascular origin^[76,80-82]. Several traditional CV risk factors (diabetes mellitus, hypertension, dyslipidemia, and smoking) are frequently found in individuals with erectile dysfunction, conferring a detrimental cardiovascular burden to them. More interestingly, the increased cardiovascular risk observed in those patients is independent of the aforementioned CV risk factors^[81-88].

A recent systematic review and meta-analysis of relevant studies in this field confirmed that erectile dysfunction is associated with increased risk of CV events and all-cause mortality^[89]. The pooled relative risks were 1.44 (95%CI: 1.27-1.63) for total CV events, 1.19 (95%CI: 0.97-1.46) for CV mortality, 1.62 (95%CI: 1.34-1.96) for myocardial infarction, 1.39 (95%CI: 1.23-1.57) for cerebrovascular events, and 1.25 (95%CI: 1.12-1.39) for all-cause mortality, for men with *vs* without erectile dysfunction. Of note, the relative risk was higher in intermediate-compared with high- or low-CV-risk populations and with younger age, with obvious clinical implications. Interestingly, the relative risks were higher when erectile dysfunction was diagnosed with the use of a questionnaire compared with a single question (RR =

1.61; 95%CI: 1.38-1.86 *vs* RR = 1.27; 95%CI: 1.18-1.37, respectively; *P* = 0.006).

Since erectile dysfunction presents such an intimate relationship with CV parameters, it is easily deduced that it could constitute a powerful tool for detecting asymptomatic CV disease. Consequently, recognition of sexual dysfunction in a hypertensive individual should prompt further diagnostic procedures and therapeutic interventions in order to disclose its silent cardiovascular risk and improve patient's quality of life and life expectancy.

SEXUAL ACTIVITY IN PATIENTS WITH CV DISEASE

Considering the fact that CV disease presents with higher incidence in patients with erectile dysfunction while at the same time sexual activity by itself poses potential CV risks, the appropriate management of those complex conditions is of utmost importance. Accordingly, the working group of the third Princeton Consensus Conference developed practical guidelines and a simplified algorithm in order to manage sexual dysfunction and sexual activity implementation issues in patients with different levels of CV risk, including hypertensive patients^[90].

In particular, patients are classified into three categories (low, intermediate, high) depending on their CV risk profile. Individuals with controlled hypertension belong to the low-risk group where sexual dysfunction can be safely managed with the approved medical therapies regardless of the number or class (with the exception of β -blockers and diuretics) of agents of the patient's antihypertensive regime. Moreover, patients of this group can safely initiate or reinstitute sexual activity without any need for additional cardiovascular evaluation.

On the contrary, patients with uncontrolled hypertension (poorly controlled, untreated, accelerated or malignant) belong to the high risk group where both treatment of sexual dysfunction and sexual activity resumption must be deferred until a thorough and specialized evaluation and stabilization has primarily been made.

Erectile dysfunction usually precedes cardiovascular events by 3 to 5 years. Therefore, sexual function should be incorporated into cardiovascular disease risk assessment for all men. Recently, algorithms for the management of patients with erectile dysfunction according to the risk for sexual activity and future cardiovascular events were proposed^[91]. A comprehensive approach to cardiovascular risk reduction (comprising of both lifestyle changes and pharmacological treatment) will result in significant benefits on overall vascular health, including sexual function. Proper sexual counselling will exert beneficial effects on the quality of life of hypertensive patients with erectile dysfunction and will improve adherence to antihypertensive drug therapy^[91].

CONCLUSION

The prevalence of erectile dysfunction is approximately

2-fold higher in hypertensive patients compared to normotensive individuals. However, erectile dysfunction remains under-reported, under-recognized, and under-treated in hypertensive patients. Hypertension *per se* and antihypertensive drug therapy may contribute to the development of erectile dysfunction in patients with arterial hypertension. The management of erectile dysfunction in hypertensive patients is tricky and should take into account the different effects of antihypertensive drug categories on erectile function. Lifestyle modification should be the mainstay of treating erectile dysfunction in patients with untreated hypertension. Switching antihypertensive therapy should be considered in treated hypertensive patients, unless administered drugs are absolutely indicated for the individual patient. Otherwise, PDE-5 inhibitors should be used, since they are both effective and safe in hypertensive patients. Finally, erectile dysfunction offers the opportunity to recognize asymptomatic cardiovascular disease and better characterize the relevant risk with obvious benefits for cardiovascular disease prevention.

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WJC 6th Anniversary Special Issues (3): Cardiomyopathy

Multimodality imaging in apical hypertrophic cardiomyopathy

Rosario Parisi, Francesca Mirabella, Gioel Gabrio Secco, Rossella Fattori

Rosario Parisi, Francesca Mirabella, Gioel Gabrio Secco, Rossella Fattori, Interventional Cardiology, AO Ospedali Riuniti Marche Nord, 61121 Pesaro, Italy

Author contributions: All authors contributed to the production of the manuscript.

Correspondence to: Rossella Fattori, MD, PhD, Interventional Cardiology, AO Ospedali Riuniti Marche Nord, Piazzale Cinelli 1, 61121 Pesaro, Italy. rossella.fattori@unibo.it

Telephone: +39-7-21362683 Fax: +39-7-21362291

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Abstract

Apical hypertrophic cardiomyopathy (AHCM) is a relatively rare morphologic variant of HCM in which the hypertrophy of myocardium is localized to the left ventricular apex. Symptoms of AHCM might vary from none to others mimic coronary artery disease including acute coronary syndrome, thus resulting in inappropriate hospitalization. Transthoracic echocardiography is the first-line imaging technique for the diagnosis of hypertrophic cardiomyopathies. However, when the hypertrophy of the myocardium is localized in the ventricular apex might results in missed diagnosis. Aim of this paper is to review the different imaging techniques used for the diagnosis of AHCM and their role in the detection and comprehension of this uncommon disease.

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Key words: Apical hypertrophic cardiomyopathy; Imaging techniques; Cardiac magnetic resonance; Transthoracic echocardiography; Multidetector computed tomography

Core tip: Apical hypertrophic cardiomyopathy (AHCM) is a relatively rare morphologic variant of HCM in which the hypertrophy of myocardium is localized to the left ventricular apex. Aim of this paper is to review the dif-

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a genetic disorder caused by mutations in one or more of the genes encoding protein components of the cardiac sarcomere and transmitted with an autosomal dominant trait and variable penetrance^[1,2]. The variability of these mutations leads to different morphological features of the pathology and influences patient prognosis^[3,4].

Apical HCM (AHCM) is a relatively rare morphologic variant of HCM in which the hypertrophy of the myocardium is mainly localized to the left ventricular (LV) apex without the typical septal predominance, which characterize hypertrophic obstructive cardiomyopathy. A sarcomere protein gene defects have been found to be present from 13% to 30% of these patients^[5]. It was first described in Japanese patients with precordial deep T wave inversions (referred to as giant T wave inversions) in 1976^[6,7]. This condition is frequent in Asian population accounting for almost 25% of Japanese patients with HCM while its prevalence dramatically decrease in Caucasian patients to 1%-3%^[8-10]. Male gender is the most frequently affected in the Japanese population but this gender difference has not been as relevant outside Japan^[11]. Differences between the “pure” Japanese form of AHCM (hypertrophy of only the apical segments) and the non-Japanese form are reported. AHCM in Caucasian patients presents hypertrophy extended to the middle

Table 1 Comparison of different imaging techniques

	Echocardiography	SPECT	Angiography	MDCT	CMR
LV morphology (dimensions, wall thickness)	++	-	+	++	+++
Global and regional LV function	++	+	+	++	+++
LV filling pressure	++	+	+++	+	++
Radiation	-	+	+	+	-
Ischemia/CAD	+	++	+++	++	++
Tissue characterisation	+	-	-	-	+++
Cost	-	+++	+++	++	++

SPECT: Single photon emission computed tomography; MDCT: Multidetector computed tomography; CMR: Cardiovascular magnetic resonance; LV: Left ventricular; CAD: Coronary artery disease.

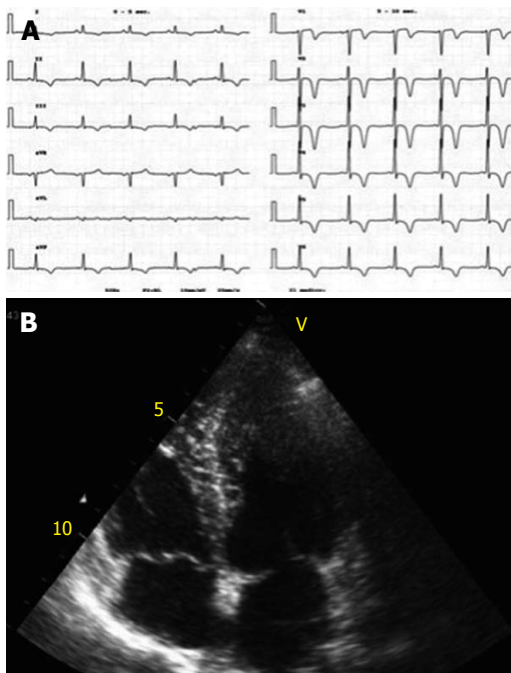


Figure 1 On transthoracic echocardiography, apical hypertrophic cardiomyopathy is defined as an absolute apical thickness of more than 15 mm with a ratio of apical to basal left ventricular wall thickness of more than 1.3. A: 12-lead electrocardiogram with increased in QRS voltage and deep T-wave inversion in the precordial leads; B: Transthoracic echocardiography 4-chambers view showing asymmetrical left ventricular apical thickening with a spade shaped left ventricle configuration.

left ventricle's segment segments ("mixed form"), with a worsened prognosis. These findings suggest a variability in the phenotypic expression of AHCM between countries and races with a possible additional role of environmental factors^[12,13].

AHCM has a relatively benign prognosis in terms of cardiovascular mortality ranging around 0.1% in "pure" forms. However, one-third of the patients may experience unfavourable clinical events and life treating complications: diastolic dysfunction, myocardial infarction, left atrial enlargement with subsequent atrial fibrillation, apical aneurysm and thrombi with ventricular arrhythmias^[10,12]. Moreover, progression into apical aneurysm or mid-ventricular obstruction is a variant and unfavourable feature of the disease. Symptoms might vary from none to others including chest pain in absence of angiographi-

cally proven coronary stenosis, palpitations, dyspnea, fatigue or syncope^[14]. ECG pattern found in up to 90% of cases, include giant negative T waves at rest with transient normalization on exertion. Transthoracic echocardiography is currently the standard diagnostic tool for hypertrophic cardiomyopathies, however its diagnostic accuracy for identification of hypertrophy confined to the LV apex is limited.

Aim of this paper is to briefly review the different imaging techniques in the diagnosis of AHCM and their potential role in expanding our knowledge of this uncommon disease (Table 1).

ECHOCARDIOGRAPHY

Transthoracic echocardiography (TTE) is the first line imaging exam in patient with suspected AHCM because of its widespread availability and low-cost. On TTE, AHCM is defined as an absolute apical thickness of more than 15 mm with a ratio of apical to basal LV wall thickness of more than 1.3 (Figure 1). According to patterns of hypertrophy, two morphologically distinct phenotypes have been described: pure AHCM where the hypertrophy is limited to the apical segments and mixed AHCM with hypertrophy extending to the mid-ventricular level, sparing the basal segments^[15]. Morphological subtypes have been found to be predictors of different prognosis and clinical manifestations^[16]. Tissue Doppler technique enables to document a lowered coronary flow reserve capacity of penetrating intramyocardial coronary arteries^[17]. However, because of technical artefacts and variability of imaging quality, TTE might results in poor detection of endocardial border thus resulting in misleading diagnosis^[18]. Patients with AHCM might develop apical aneurysms and clots mimicking other conditions such as cardiac tumor, isolated ventricular non-compaction, endomyocardial fibrosis, *etc.* The use of microbubbles contrast agent may improve diagnostic sensitivity^[19-23].

Newer Doppler-based techniques have been successfully applied in the diagnosis of AHCM. Reddy *et al*^[24] described paradoxical apical longitudinal strain (systolic lengthening) in two patients with AHCM despite an apparently normal apical wall motion on conventional TTE. Abecasis *et al*^[25] using velocity vector imaging tissue characterization study found abnormal regional velocities and

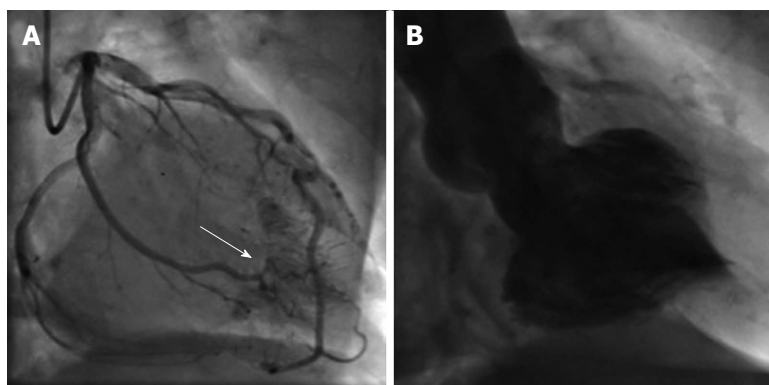


Figure 2 Angiography pictures. A: Coronary angiography showing normal epicardial coronary arteries. Please note the presence of multiple coronary artery-left ventricular microfistulae (white arrow); B: Left ventricular angiography showing the characteristic diastolic “ace-of-spade” sign.

deformation parameters, particularly concerning base to apex longitudinal strain gradient, that could be related to the abnormal tissue hypertrophy extending beyond the more evident apical hypertrophic segments.

Multipane transoesophageal echocardiography enables a correct visualization and sizing of ventricular segments and has been successfully applied in the diagnosis of AHCM^[26].

SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY

Radionuclide scanning has also been used in diagnosis of AHCM. Reports of stress myocardial perfusion images in patients with AHCM have ranged from normal perfusion to reversible and fixed apical perfusion defects, often in the presence of normal epicardial coronary arteries^[27]. The unbalanced wall thickness-to-vascular supply ratio leads to a relative apical ischemia^[28,29]. Myocardial ischemic chest pain in the absence of coronary artery disease (CAD) has been related to limited coronary flow reserve in patients with asymmetric septal an apical hypertrophy^[29-32]. Morishita *et al*^[33] have also described increased uptake of Tc-99 m tetrofosmin in the apical segment on resting Single Photon Emission Computed Tomography (SPECT) polar maps in a subgroup of patients with AHCM. AHCM increased apical tracer uptake on resting Tl-201 planar and SPECT imaging has been previously reported^[34]. Ward *et al*^[35] showed a newly “Solar Polar” map pattern at rest. This “Solar Polar” map pattern on resting Tl-201 volume-weighted polar maps, sees an intensely bright spot of counts in the apical segment surrounded by a circumferential ring of decreasing counts. This study is the first describing the typical findings on dual-isotope rest and stress SPECT perfusion images and volume-weighted polar maps in non-Japanese patients with AHCM. Three different patterns characteristic of AHCM were identified^[36]: an increased apical tracer uptake, a spade-like configuration of the LV chamber and the “Solar map” in 75% of patients; however no difference in apical thickness and magnitude of T-wave negativity between patients with normal SPECT and typical

pattern were observed. Interstitial fibrosis that prevented the increased apical tracer uptake is the possible explanation for a normal SPECT study in patients with AHCM.

ANGIOGRAPHY

ECG changes and symptoms associated with AHCM often mimic acute coronary syndromes. Moreover elevated troponine serum levels reported in patients with AHCM and chest pain usually encourage physicians to perform invasive testing. Coronary angiography allows to exclude significant epicardial coronary lesions and enables detection of the associated congenital coronary artery anomalies, myocardial bridge or multiple coronary-LV fistulae^[37]. Evaluation of the LV cavity can show the characteristic spade-like configuration of the left ventricle in end-diastole, with obliteration of the apical cavity in end-systole due to the vigour contraction of the hypertrophied myocardium^[7] (Figure 2). Caucasian patients tend to have less localized involvement of the distal apex resulting in a lower frequency of the pathognomonic sign of “ace-of-spade” on the left ventriculography^[13].

MULTIDETECTOR COMPUTED TOMOGRAPHY

Coronary multidetector computed tomography (MDCT) is an high sensitive (91%-99%) and specific (74%-96%) technique in detecting significant coronary stenosis^[38-40]. Major international guidelines currently indicate coronary MDCT for patients at a low to intermediate risk of CAD^[41] and his adoption in the emergency room might facilitate early triage of patients presenting chest pain^[42-44].

MDCT has also emerged as a novel technique for evaluating cardiac morphology and function. Initial concern with MDCT examination with radiation exposure have been overcome by novel technologies using dose-saving strategies^[45,46]. Due to its high spatial resolution, MDCT can offer cardiac anatomical and functional information and a high quality non-invasive coronary evaluation^[47-50]. It also enables accurate delineation of the apical endocardial border and dynamic evaluations of myocar-

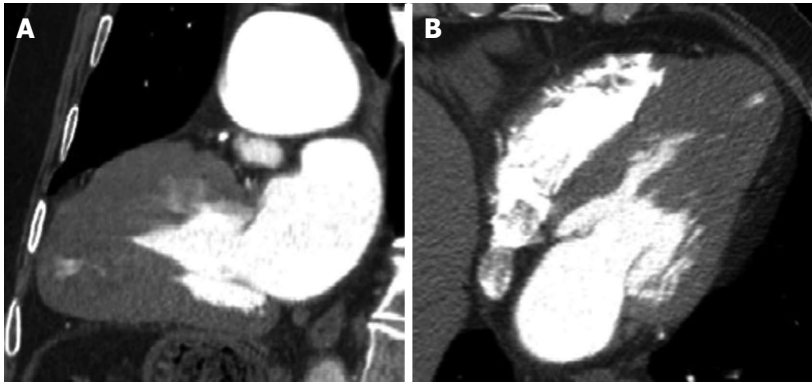


Figure 3 Multidetector computed tomography imaging: Long axis view (A) and axial scans at the level of the left ventricle (B) showing apical hypertrophy with cavity obliteration and a sequestered small left ventricle cavity.

dial thickness, global and regional LV functions^[51]. Multi-planar reconstructions along major cardiac axis allow to measure myocardial thickness on short-axis view in the end-diastolic phase while the apex can be evaluated in long axis planes (Figure 3). Knickelbine *et al*^[52] have found nonatherosclerotic-related cardiovascular abnormalities judged to be of potential clinical relevance in 4.4% of 4543 patients with suspected atherosclerotic CAD undergoing to 64-slice MDCT. In 50 of these patients (1.1%) the abnormality was previously unrecognized. The most common abnormalities were: congenital coronary artery anomalies (38%), ascending aortic aneurysms > 45 mm (22%), hypertrophic cardiomyopathy with apical LV wall thickening (14%), valvular heart diseases (8%), congenital heart diseases including ventricular septal defect (6%), pulmonary embolus (6%), LV noncompaction, left atrial myxoma, and LV apical aneurysm (2%). Chen *et al*^[53] have performed MDCT in 14 patients with known diagnosis of AHCM. Left ventricle shapes reconstructions of MDCT were similar to angiography, with “ace-of-spades” configurations, apical sequestrations and apical aneurysm. Furthermore, MDCT was able to detect two cases of significant coronary stenosis and 7 patients with myocardial bridges.

CARDIOVASCULAR MAGNETIC RESONANCE

In the last few years cardiovascular magnetic resonance (CMR) has emerged as a useful and accurate imaging technique for diagnosis of HCM. Both European and American Cardiology Society indicated CMR as first choice exam or at least equivalent to other diagnostic methods in the approach of several cardiomyopathies, including HCM^[54,55].

The excellence of CMR in analyse anatomy and function has increased the sensitivity and specificity of the diagnosis of HCM^[56]. A comparative study of TTE and CMR among HCM subjects demonstrated the greater accuracy of CMR identifying different patterns of hypertrophy. Among subjects with confined hypertrophy in anterolateral wall, echocardiography underestimates wall

thickness and poorly evaluates the apical segments in up to 40%^[57-59]. AHCM may mimic other pathological conditions such as coronary artery disease, myocardial tumor, ventricular aneurysm, ventricular non-compaction or endomyocardial fibrosis and CMR can be useful in differential diagnosis. CMR provides a more accurate assessment of LV apical hypertrophy allowing detection of HCM related complications and wall motion abnormalities (Figure 4). Tsukamoto *et al*^[60] using CMR-tagging showed systolic outward motion of the LV apical wall in AHCM patients. LV apical aneurysms have been reported in up to 2% of all patients with HCM, with a rate of related adverse events of 10.5% per year, considerably higher with respect to HCM without aneurysm^[61]. Notably, a higher incidence of apical aneurysms, ranging from 10% to 20%^[62,63], has been reported in AHCM. In a case series, Fattori *et al*^[64] showed that TTE was able to detect only 1 of the 4 cases of AHCM related apical aneurysms, suggesting the use of CMR in all patients affected by AHCM in order to confirm the diagnosis and to ascertain the presence of aneurysms. Indeed, the presence of an apical aneurysm, especially if associated with the detection of ventricular tachyarrhythmias, could support the decision to implant a cardioverter-defibrillator.

CMR appears to be more sensitive than other imaging techniques in detecting infarct areas and ischemia, identifying even subendocardial infarction with late gadolinium-enhanced (LGE)^[65,66]. LGE-CMR has been used to visualize myocardial interstitial abnormalities in patients with different forms of cardiomyopathies, including non-ischemic forms^[67,68]. LGE has been found to be present in a high proportion of patients with HCM and has been associated with a higher incidence of ventricular tachycarrythmias and risk of sudden death^[69,70]. In patients with apical hypertrophic cardiomyopathy, the incidence of LGE seems to be less common with respect to other form of HCMP, but it is similarly associated to a worse prognosis. In the largest available series of AHCM patients imaged with magnetic resonance imaging, LGE was reported only in 40% of cases and limited to the hypertrophic apical segments^[71]. However, others studies showed that LGE was not limited to the hypertrophic

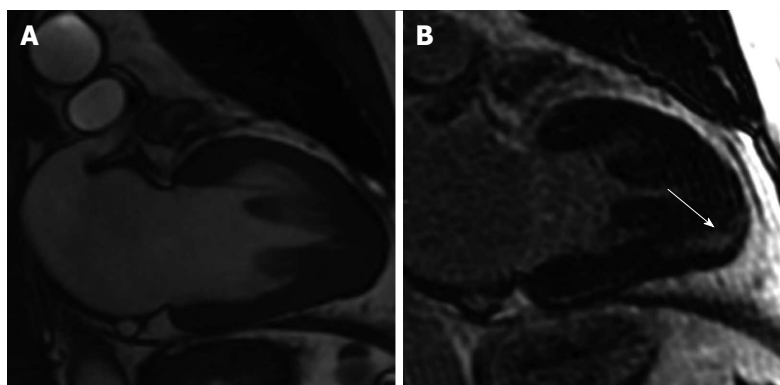


Figure 4 Cardiovascular magnetic resonance imaging. Long axis view (A) of the left ventricle showing apical regional hypertrophy; long axis view 10 min after Gadolinium injection; B: An abnormal hyper-enhancement of the apical segment is visible (white arrow).

apical segments but also present in the midventricular and basal segments of interventricular septum, potential expression of myocardial damage preceding the abnormal hypertrophy. LGE-CMR should be applied for longitudinal follow-up studies to detect development and progression of AHCM related fibrotic tissue formations highlighting the subsets of patients associated with worse prognosis^[72].

CONCLUSION

The correct diagnosis of AHCM is of major importance. Multimodality imaging is essential in increasing the detection of AHCM, yielding larger study populations. In particular, CMR showed an excellent accuracy in identifying the abnormal LV hypertrophy. With late gadolinium enhancement, CMR is able to *in vivo* detect abnormal myocardial structure allowing a more accurate risk stratification.

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WJC 6th Anniversary Special Issues (5): Myocardial infarction

Thrombus aspiration in acute myocardial infarction: Rationale and indication

Gennaro Sardella, Rocco Edoardo Stio

Gennaro Sardella, Rocco Edoardo Stio, Department of Cardiovascular, Respiratory, Nephrologic and Geriatric Sciences, "Sapienza" University of Rome, Policlinico Umberto I, 00161 Rome, Italy

Author contributions: Sardella G designed and performed the research; Stio RE wrote the paper.

Correspondence to: Gennaro Sardella, MD, FACC, FESC, Department of Cardiovascular, Respiratory, Nephrologic and Geriatric Sciences, "Sapienza" University of Rome, Policlinico Umberto I, Viale del Policlinico 155, 00161 Rome, Italy. rocco.stio@libero.it

Telephone: +39-06-49979035 Fax: +39-06-49979060

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Abstract

Reperfusion of myocardial tissue is the main goal of primary percutaneous coronary intervention (PPCI) with stent implantation in the treatment of acute ST-segment elevation myocardial infarction (STEMI). Although PPCI has contributed to a dramatic reduction in cardiovascular mortality over three decades, normal myocardial perfusion is not restored in approximately one-third of these patients. Several mechanisms may contribute to myocardial reperfusion failure, in particular distal embolization of the thrombus and plaque fragments. In fact, this is a possible complication during PPCI, resulting in microvascular obstruction and no-reflow phenomenon. The presence of a visible thrombus at the time of PPCI in patients with STEMI is associated with poor procedural and clinical outcomes. Aspiration thrombectomy during PPCI has been proposed to prevent embolization in order to improve these outcomes. In fact, the most recent guidelines suggest the routine use of manual aspiration thrombectomy during PPCI (class II a) to reduce the risk of distal embolization. Even though numerous international studies have been reported, there are conflicting results on the clinical impact of aspiration throm-

bectomy during PPCI. In particular, data on long-term clinical outcomes are still inconsistent. In this review, we have carefully analyzed literature data on thrombectomy during PPCI, taking into account the most recent studies and meta-analyses.

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Key words: Thrombus aspiration; Thrombectomy; Myocardial reperfusion; Myocardial infarction; No-reflow

Core tip: Distal coronary embolization occurs predominantly at the time of the initial balloon or stent inflation, so thrombus burden reduction by thrombectomy devices before percutaneous coronary intervention may decrease the dangerous phenomenon of no-reflow. Manual aspiration catheters are the most commonly used devices. Several randomized trials have demonstrated the efficacy and safety of pretreatment with manual thrombectomy during primary percutaneous coronary intervention. There are some unanswered questions about thrombus aspiration, including whether there is truly a mortality benefit, which subgroups may or may not benefit from aspiration, and whether patients with a large thrombus burden are better treated with mechanical thrombectomy.

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INTRODUCTION

The final objective of primary percutaneous coronary intervention (PPCI) is successful myocardial reperfusion^[1]. Apart from restoration of flow in the epicardial coronary

artery, the importance of cardiac muscle microcirculation has been emphasized^[2,3]. Myocardial reperfusion failure has been associated with larger infarct size, increased predisposition to ventricular arrhythmias, heart failure, cardiogenic shock, recurrent myocardial infarction, and cardiac death^[4,5].

Different mechanisms are responsible for microvascular injury after PPCI, such as local formation of a thrombus, generation of oxygen-free radicals, myocyte calcium overload, cellular and interstitial edema, endothelial dysfunction, vasoconstriction, and inflammation. However, distal embolization seems to play a pivotal role, and thrombus burden is a predictor of the no-reflow phenomenon and an independent predictor of adverse outcomes^[6-10].

Distal coronary embolization occurs predominantly at the time of initial balloon or stent inflation, so thrombus burden reduction by thrombectomy devices before balloon/stent inflation may decrease the dangerous phenomenon of no-reflow^[11]. Manual aspiration catheters are the most commonly used devices because they are easy and safe to use, even in the elderly^[12], and are relatively inexpensive compared with rheolytic thrombectomy^[13]. Moreover, myocardial salvage is measured and studied in trials through different parameters: angiographic [thrombolysis in myocardial infarction (TIMI) and myocardial blush grade (MBG)], electrocardiographic [ST-segment resolution (STR)], functional (reduction of infarct size) and clinical (enhanced survival free from heart failure events)^[14,15]. Several randomized trials have demonstrated the efficacy and safety of pretreatment with manual thrombectomy during PPCI. Most of the studies in the literature, including meta-analyses, randomized trials or registries, conclude that thrombectomy improves the parameters of myocardial reperfusion, with a rapid and effective STR^[16]. The Thrombus Aspiration during Percutaneous coronary intervention in Acute myocardial infarction (TAPAS) Trial, the impact of thrombectomy with EXPort catheter in Infarct-Related Artery during Primary Percutaneous Coronary Intervention (EXPIRA) Trial and some meta-analyses found that aspiration thrombectomy during ST-segment elevation myocardial infarction (STEMI) improves myocardial reperfusion and procedural outcomes, reducing no-reflow, mortality and distal embolization^[17-19]. There are some unanswered questions about thrombus aspiration including whether there is truly a mortality benefit^[20], which subgroups may and may not benefit from aspiration, and whether patients with large thrombus burden are better treated with mechanical thrombectomy.

RATIONALE AND INDICATION

There are many ways to treat the coronary thrombus burden at the time of PPCI: pharmacologic strategies (typically glycoprotein II b/IIIa platelet inhibitors), embolic protection devices (filters and distal balloon occlusion with aspiration), mechanical thrombectomy, and manual

or aspiration thrombectomy devices. This paper reviews the role of manual thrombectomy in patients with STEMI. The evidence supporting the benefit of aspiration thrombectomy on surrogate outcomes (TIMI flow, MBG and STR) and angiographic outcomes (distal embolization and no-reflow) is strong and convincing, while the benefit in reduction of mortality is not strong and has limitations^[19-24].

All randomized trials of aspiration thrombectomy have been performed in "all comers" with STEMI, and it is not clear which subgroups may benefit more and which subgroups may not benefit at all. In the EXPIRA Trial, 175 patients with STEMI were randomized to PPCI alone *vs* PPCI with manual thrombectomy and a significant improvement was shown in the primary endpoints of MBG 3 and complete STR. This study was the first to evaluate infarct size by magnetic resonance imaging, and it found that the extent of microvascular obstruction was less in the acute phase with aspiration (1.7 g *vs* 3.7 g, $P = 0.0003$), and an improvement in infarct size at 3 mo was seen with aspiration (17% to 11%, $P = 0.004$) but not in the control group (14% to 13%, $P = \text{NS}$)^[18]. These data are confirmed by the results of the INFUSE-AMI Trial (Intracoronary Abciximab Infusion and Aspiration Thrombectomy in Patients Undergoing Percutaneous Coronary Intervention for Anterior ST-Segment Elevation Myocardial Infarction) in which the group with thrombectomy plus intracoronary abciximab had a better prognosis^[25].

Based on the TAPAS Trial and the above meta-analyses, the American College of Cardiology/American Heart Association Guidelines and the European Society of Cardiology Guidelines have given aspiration thrombectomy a Class IIa (Level of Evidence B) indication in PPCI for STEMI. The committee did not consider the evidence for benefit on clinical outcomes strong enough to warrant a Class I indication^[22].

The literature and clinical practice clearly show that the impact of thrombectomy on all outcomes is linked to multiple factors during STEMI, in particular time from symptom onset to PCI, and infarct-related coronary artery and intracoronary thrombus burden.

Sianos *et al*^[26] have shown that both angiographic and clinical outcomes are poorer in patients with a large thrombus burden (≥ 2 vessel diameters) in a new thrombus classification. A large thrombus burden is associated with a greater frequency of major adverse cardiac events, and is a strong independent predictor of late mortality. Moreover, Napodano *et al*^[27] found that patients with right coronary artery infarcts, long lesions and a high thrombus score had the highest frequency of distal embolization. We might expect these subgroups to benefit most from thrombectomy, but data from the TAPAS trial do not support this. Improvement in MBG with aspiration was no better in patients with right coronary artery (RCA) infarcts *vs* non-RCA infarcts, and was no better in patients with a visible thrombus compared with patients without a visible thrombus. There was a trend for greater

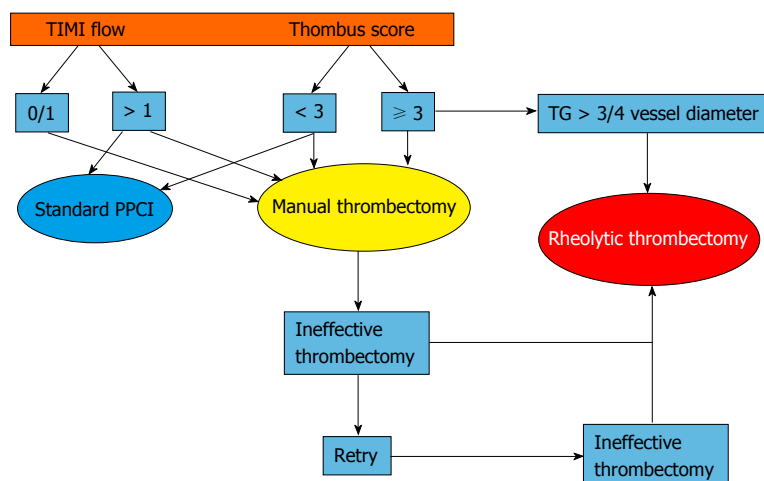


Figure 1 The pathway indicated by the green arrow is recommended during primary percutaneous coronary intervention. PPCI: Primary percutaneous coronary intervention; TIMI: Thrombolysis in myocardial infarction.

benefit in patients with a reperfusion time of less than 3 h, but there were no differential benefits in patients stratified by pre-PCI TIMI flow^[17]. Overall, there are few current studies to support selective use of aspiration thrombectomy in any subgroup of STEMI patients treated with PPCI^[28-30].

Recently, the TASTE Trial (Thrombus Aspiration in ST-Elevation myocardial infarction in Scandinavia), a randomized study using a platform of a clinical registry, enrolled 7244 STEMI patients who were treated with standard PPCI or manual thrombectomy before PCI. This trial had an ambitious primary endpoint, that is, to reduce 30-d all-cause mortality, and it concluded that routine thrombectomy in PPCI does not reduce this event^[31]. In our opinion, in this study, it was excessive to expect a mortality reduction at 30 d, and would have been more logical to have a primary end-point with a mean follow-up of at least 1 year, as in TAPAS. The TASTE trial design was based on national heart registries and on a secondary randomization that could introduce an initial bias; moreover, there were no reported procedural data such as TIMI flow post-aspiration, MBG or STR. Finally, the frequency of thrombus score greater than 3 was very low (32%) in the total population (54% of patients in the TASTE trial). Instead in the EXPIRA trial, an important inclusion criteria was a higher visible thrombus burden (score ≥ 3) identifying patients at highest risk of coronary distal embolization. Data reported in the literature and guidelines indicate that manual thrombus aspiration should always be considered during PPCI to reduce the risk of distal embolization, in particular in cases of intraluminal thrombosis with a score ≥ 3 .

Aspiration thrombectomy has limited ability to remove a large thrombus and may sometimes be associated with incomplete thrombus removal, no-reflow, and/or distal emboli. There is previous and very recent evidence that mechanical thrombectomy may effectively improve outcomes in patients with a large thrombus burden. Whether mechanical thrombectomy is preferable to aspiration thrombectomy in patients with a large thrombus

burden remains an unanswered question^[32].

CONCLUSION

In our opinion, based on the literature and clinical practice, manual thrombectomy can be used as first approach during PPCI to prevent distal embolization in the case of a visible thrombus burden. As demonstrated in the RET-AMI trial, the new generation manual thrombectomy devices are superior to the first generation tools to remove a greater thrombotic burden, providing higher post-thrombectomy epicardial flow and better post-stenting microvascular reperfusion^[33].

From a “real world point of view”, to perform a good manual thrombectomy, the culprit vessel diameter could be > 2.5 mm with a TIMI flow 0-1 and a visible thrombus (score > 3). The device, however, has to advance delicately over the thrombotic occlusion to perform continuous intracoronary blood suction. In the case of a large thrombus burden, it is now possible to use a 7 Fr intracoronary manual thrombectomy device or rheolytic tools with greater suction force (Figure 1). In conclusion, in the treatment of acute myocardial infarction, thrombectomy should be considered as one of the most important therapeutic tools, with the purpose of cardioprotection and myocardial salvage.

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Drug-eluting stents and acute myocardial infarction: A lethal combination or friends?

Shuji Otsuki, Manel Sabaté

Shuji Otsuki, Manel Sabaté, Thorax Institute, Department of Cardiology, Hospital Clinic, University of Barcelona, 08015 Barcelona, Spain

Author contributions: All authors contributed to this work.

Correspondence to: Manel Sabaté, MD, PhD, Thorax Institute, Department of Cardiology, Hospital Clinic, University of Barcelona, C/Villarroel 170, 08036 Barcelona, Spain. masabate@clinic.ub.es

Telephone: +34-93-2275400 Fax: +34-93-2279305

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Abstract

Primary percutaneous coronary intervention is the preferred reperfusion strategy for patients presenting with ST-segment elevation myocardial infarction (STEMI). First generation drug-eluting stents (DES), (sirolimus drug-eluting stents and paclitaxel drug-eluting stents), reduce the risk of restenosis and target vessel revascularization compared to bare metal stents. However, stent thrombosis emerged as a major safety concern with first generation DES. In response to these safety issues, second generation DES were developed with different drugs, improved stent platforms and more biocompatible durable or bioabsorbable polymeric coating. This article presents an overview of safety and efficacy of the first and second generation DES in STEMI.

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Key words: ST-segment elevation myocardial infarction; Drug-eluting stents; Stent thrombosis; Sirolimus drug-eluting; Paclitaxel drug-eluting stents; Zotarolimus-eluting stents; Zotarolimus-eluting stents; Bioresorbable vascular scaffold

Core tip: Primary percutaneous coronary intervention is the preferred reperfusion strategy for patients present-

ing with ST-segment elevation myocardial infarction (STEMI). First-generation drug-eluting stents (DES) reduce restenosis and target vessel revascularization compared to bare metal stents at the expense of an increased stent thrombosis rate. Recent improvements in second-generation DES have overcome these safety concerns. This article presents an overview of safety and efficacy of the DES in STEMI.

Otsuki S, Sabaté M. Drug-eluting stents and acute myocardial infarction: A lethal combination or friends? *World J Cardiol* 2014; 6(9): 929-938 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i9/929.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i9.929>

INTRODUCTION

Primary percutaneous coronary intervention (PCI) has become a well-established reperfusion strategy for patients presenting with acute ST-segment elevation myocardial infarction (STEMI)^[1,2]. In this setting, bare-metal stents (BMS) reduced the risk of recurrent ischemia and restenosis compared to balloon angioplasty^[3]. First-generation drug-eluting stents (DES)-sirolimus-eluting stents (SES) and paclitaxel-eluting stents (PES)-were also able to reduce the risk of restenosis and target-vessel revascularization (TVR) compared to BMS in this context^[4,5]. However, stent thrombosis emerged as a major safety concern^[6]. In response, second-generation DES were developed with different drugs, more biocompatible durable polymers or bioabsorbable polymeric coatings, and new stent platforms, including fully bioresorbable vascular scaffolds.

PATHOPHYSIOLOGY OF STEMI

As shown in Figure 1, STEMI is an event related to ath-

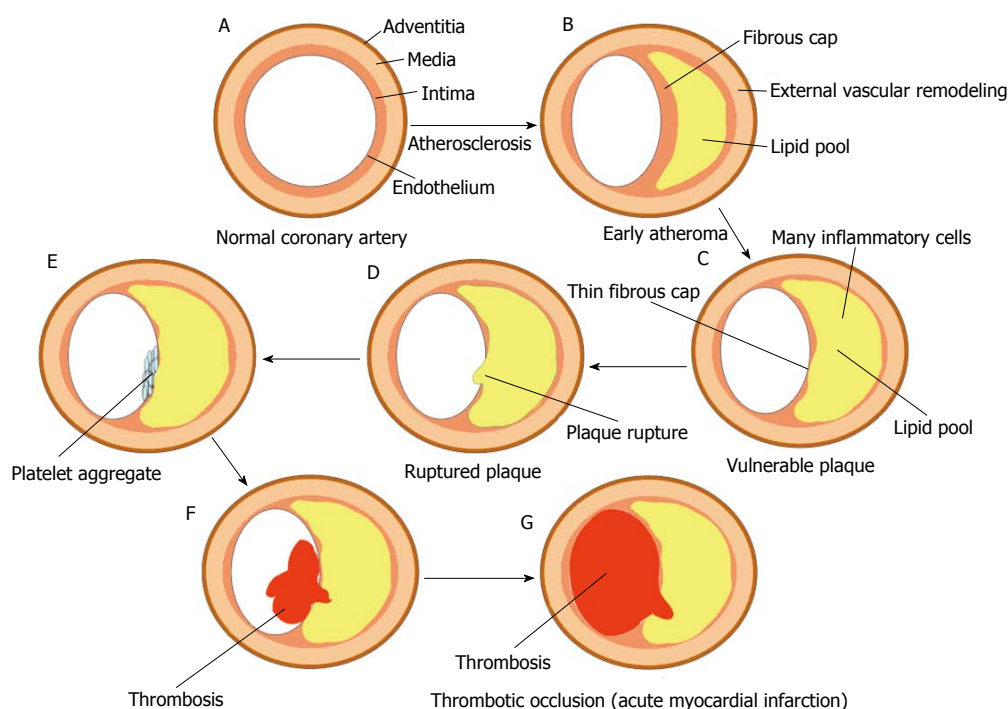


Figure 1 Pathophysiology of ST-segment elevation myocardial infarction. A: Normal coronary artery; B: Coronary artery with early atheroma; C: Vulnerable plaque with thin fibrous cap; D: Ruptured plaque; E: Platelets aggregated to heal the ruptured plaque; F: Protruding thrombus; G: Thrombotic occlusion (acute myocardial infarction).

erosclerotic plaque rupture, ulceration, fissuring, erosion, or dissection that results in intraluminal thrombus in one or more of the coronary arteries, leading to decreased myocardial blood flow or distal platelet emboli with ensuing myocyte necrosis^[7,8]. During the early years after the introduction of coronary stents, it was thought that implanting a metallic device under a thrombotic environment in the acute phase of STEMI could increase the risk of adverse outcome. However, refinement of stent implantation technique and the development of new anti-thrombotic regimen have overcome those initial concerns.

PATHOPHYSIOLOGY OF STENT THROMBOSIS

The pathophysiology of stent thrombosis includes procedure-, stent-, and patient-related factors (Figure 2). The PCI procedure for acute coronary syndrome, including STEMI, is one of the most powerful predictors for stent thrombosis in the vast majority of registries^[9-14] (Figure 3). Late stent malapposition is common in STEMI patients and may eventually provoke stent thrombosis. Late malapposition may be linked to underdeployment of stents at the time of STEMI treatment, due mainly to dissolution of thrombus behind the struts or undersized vessels due to the spastic condition of the coronary arteries in the acute phase of STEMI^[15]. Implanting DES over a necrotic core may also significantly delay healing, due to less neointimal growth and greater inflammation, fibrin deposits, and uncovered struts compared to DES implanted over coronary stable plaques^[16,17].

Currently, patients are categorized as having early or late stent thrombosis. Early stent thrombosis is defined as occurring within 30 d of implantation, and is further categorized as acute (events within 24 h) or subacute (events on day 1-30) thrombosis. Events that occur more than 30 d postimplantation are classified as late stent thrombosis, and those occurring beyond 12 mo as very late stent thrombosis^[18].

Early and late stent thrombosis differ in their pathophysiology and mechanism. Early stent thrombosis is mainly related to one or more procedural characteristics, such as stent underexpansion, incomplete stent apposition, dissection, thrombus, tissue protrusion, and persistent slow flow. It may occur after either BMS or DES implantation.

Late stent thrombosis may result when neointimal healing is delayed, as this can lead to inadequate neointimal coverage and/or to incomplete stent apposition. Evaluation of angiography, optical coherence tomography, and autopsy revealed that first-generation DES are associated with delayed arterial healing due to hypersensitive reactions to polymers that cause chronic inflammation^[9,16]. These phenomena are typically observed more than 1 year after implantation.

SAFETY AND EFFICACY OF FIRST-GENERATION DES IN STEMI

Twelve randomized controlled trials (RCTs) of first-generation DES outcomes in STEMI have been published^[14,5,19-33]. Comparisons were made as follows: BMS

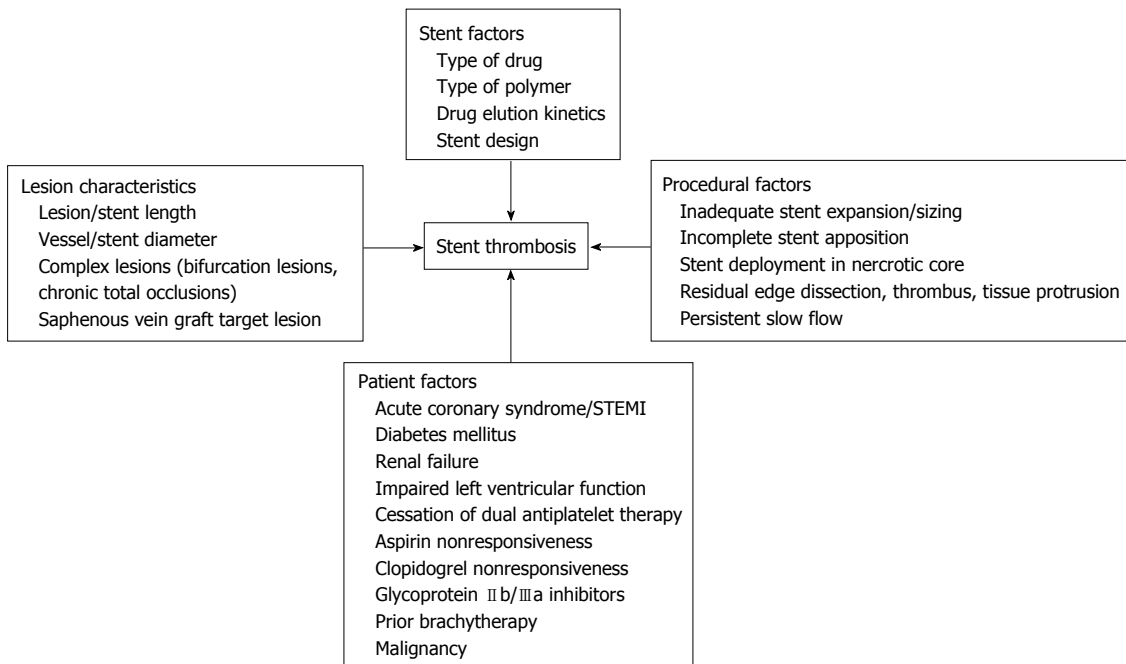


Figure 2 Potential causes of stent thrombosis. STEMI: ST-segment elevation myocardial infarction.

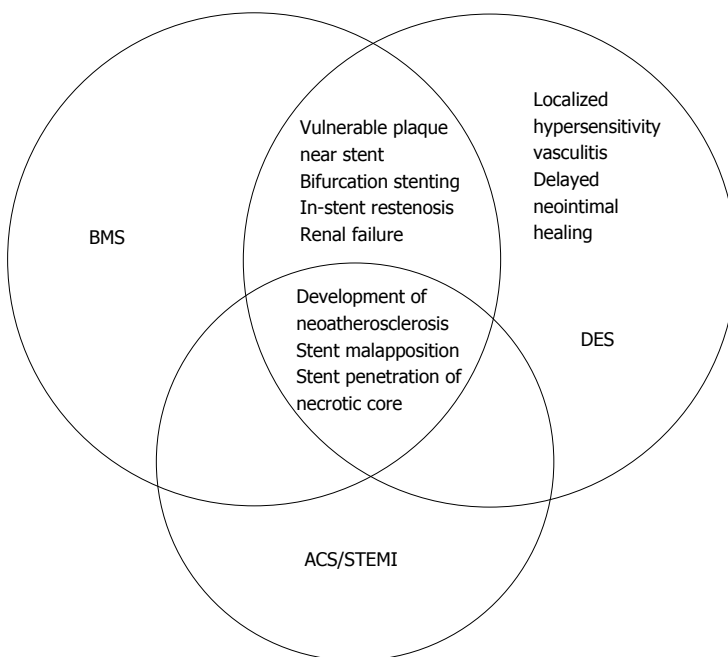


Figure 3 Multifactorial causes of late stent thrombosis. BMS: Bare metal stents; DES: Drug eluting stents; ACS: Acute coronary syndrome; STEMI: ST elevated myocardial infarction.

vs SES, 7 reports; BMS *vs* PES, 5 reports; PES *vs* SES, 2 reports; BMS *vs* SES *vs* PES, 1 report (Table 1).

The TYPHOON study^[4] was the largest RCT to consider SES, enrolling 712 patients to assess the effectiveness and safety of SES *vs* BMS at 1 year. Target-vessel failure was significantly lower in the SES (7.3%) than in the BMS (14.3%) group ($P = 0.004$), driven by a decrease in the rate of TVR (5.6% *vs* 13.4%, respectively; $P < 0.001$). There was no significant difference between the two groups in the rates of mortality (2.3% *vs* 2.2%; $P = 1.00$), repeat myocardial infarction (MI) (1.1% *vs* 1.4%; P

$= 1.00$), or stent thrombosis (3.4% *vs* 3.6%; $P = 1.00$). At 4-year follow-up^[4], freedom from target lesion revascularization was significantly better in the SES group, compared to BMS (92.4% *vs* 85.1%; $P = 0.002$). However, no differences were observed, respectively, in freedom from cardiac death (97.6% *vs* 95.9%; $P = 0.37$), freedom from repeat MI (94.8% *vs* 95.6%; $P = 0.85$), or definite/probable stent thrombosis (4.4% *vs* 4.8%, $P = 0.83$). Other studies have also reported that SES was superior or non-inferior to BMS in mortality, repeat MI, TVR, and stent thrombosis rates^[20-25,33] (Table 1).

Table 1 Randomized controlled trials of first-generation drug eluting stents in stent thrombosis elevated myocardial infarction

Study, author (Ref.)	Year	Primary endpoint	Design	Randomized ratio	Maximal length of follow-up	Stent comparators (n)	Results of the primary endpoint
Pascari <i>et al</i> ^[19]	2003	Death, MI, recurrent ischemia at 1 yr	Single center	1:1	1 yr	BMS/SES 65 (33/32)	No significant differences between stents
TYPHOON ^[4]	2006	TVF at 1 yr	Multicenter, superiority	1:1	4 yr	BMS/SES 712 (355/357)	SES superior to BMS
STRATEGY ^[20]	2007	Death, MI, stroke, binary restenosis at 8 mo	2-center, superiority	1:1	2 yr	BMS/SES 175 (87/88)	SES superior to BMS
SFESAMI ^[21,22]	2007	Binary restenosis at 1 yr	Single-center, superiority	1:1	5 yr	BMS/SES 320 (160/160)	SES superior to BMS
Díaz de la Llera <i>et al</i> ^[23]	2007	Death, MI, TLR at 1 yr	Single center, superiority	1:1	1 yr	BMS/SES 114 (54/60)	SES superior to BMS
MISSION ^[24]	2008	In-segment late luminal loss at 9 mo	Single center, noninferiority	1:1	5 yr	BMS/SES 310 (152/158)	SES superior to BMS
MULTISTRATEGY ^[25]	2008	Death, MI, clinically driven TVR at 8 mo	Multicenter, superiority	1:1	3 yr	BMS/SES 744 (372/372)	SES superior to BMS
HAAMU-STENT ^[26]	2006	Death, MI, late lumen loss, TVR at 1 yr	Single center, superiority	1:1	1 yr	BMS/PES 164 (82/82)	PES superior to BMS
SELECTION ^[27]	2007	Neointimal proliferation by IVUS at 7 mo	Single-center, superiority	1:1	7 mo	BMS/PES 76 (39/37)	PES superior to BMS
PASSION ^[28]	2008	Cardiac death, MI, TLR at 2 yr	2-center, superiority	1:1	5 yr	BMS/PES 619 (310/309)	Superiority not demonstrated
HORIZONS-AMI ^[5,29]	2009	Death, MI, stroke, or ST at 1 yr	Multicenter, superiority (TLR) Noninferiority (Death, MI, stroke, ST)	3:1	3 yr	BMS/PES 3006 (2257/749)	PES superior for TLR and noninferior for clinical endpoints
GRACIA-3 ^[30]	2010	In-segment binary restenosis, myocardial flow at 1 yr	Multicenter, noninferiority	1:1	1 yr	BMS/PES 419 (210/209)	BMS noninferior to PES
PROSIT ^[31]	2008	Death, MI, TVR, ST at 1 yr	Multicenter, superiority	1:1	3 yr	PES/SES 308 (154/154)	Superiority not demonstrated
Juwana <i>et al</i> ^[32]	2009	Late lumen loss at 9 mo	Single center, superiority	1:1	1 yr	PES/SES 397 (196/201)	SES superior to PES
PASEO ^[33]	2009	TLR at 12 mo	Single-center, superiority	1:1:1	4 yr	BMS/PES/SES 270 (90/90/90)	PES and SES superior to BMS

MI: Myocardial infarction; TLR: Target lesion revascularization; ST: Stent thrombosis; PES: Paclitaxel-eluting stents; SES: Sirolimus-eluting stents; BMS: Bare metal stent stents; TVR: Target vessel revascularization; IVUS: Intra-vascular ultrasound.

With regard to PES, the HORIZONS-AMI study was the largest RCT^[5]. A total of 3006 patients were enrolled in this 12-mo trial to assess the effectiveness and safety of PES *vs* BMS. The PES group had significantly lower 12-mo rates of ischemia-driven target lesion revascularization (4.5% *vs* 7.5%; *P* = 0.002) and TVR (5.8% *vs* 8.7%; *P* = 0.006). There were no significant differences between the PES and BMS groups in 12-mo rates of mortality (3.5% *vs* 3.5%; *P* = 0.98) and stent thrombosis (3.2% *vs* 3.4%; *P* = 0.77). At the 3-year follow-up^[29], the PES group had lower rates of ischemia-driven target lesion revascularization (9.4% *vs* 15.1%; *P* < 0.0001), but did not differ from the BMS group in mortality, repeat MI, stroke, or stent thrombosis rates. Stent thrombosis was high ($\geq 4.5\%$) in both groups. Other studies have also shown that PES was superior or noninferior to BMS in mortality, repeat MI, TVR, and stent thrombosis rates^[26,27,30,33] (Table 1).

Although RCTs did not identify any safety issues with first-generation DES, this topic became a firestorm during the 2006 European Society of Cardiology Annual Meet-

Table 2 Randomized controlled trials of second-generation drug eluting stents in ST elevated myocardial infarction

Study	Year	Primary endpoint	Design	Randomized ratio	Maximal length of follow-up	Stent comparisons (n)	Results of the primary endpoint
ZEST-AMI ^[38]	2009	Death, MI, and ischemia-driven TVR at 1 yr	Multicenter, safety study	1:1:1	1 yr	PES/SES/PC-ZES 328 (110/110/108)	No significant differences between stents
KOMER ^[39]	2011	Cardiac death, MI, ischemia-driven TLR at 1 yr	Multicenter, safety study	1:1:1	18 mo	PES/SES/PC-ZES 611 (202/204/205)	PC-ZES as safe as SES and PES
EXAMINATION ^[40,41]	2011	Death, MI, any revascularization at 1 yr	Multicenter, superiority	1:1	2 yr	CoCr-EES/BMS 1504 (751/747)	CoCr-EES superior to BMS
XAMI ^[42]	2012	Cardiac death, MI, TVR at 1 yr	Multicenter, noninferiority	2:1	1 yr	EES/SES 625 (404/221)	EES noninferior to SES
COMFORTABLE AMI ^[43]	2012	cardiac death, reinfarction, and TLR at 1 yr	Multicenter, superiority	1:1	1 yr	EES/BMS 1161 (575/582)	BES superior to BMS

MI: Myocardial infarction; TLR: Target lesion revascularization; CoCr-EES: Cobalt-chromium everolimus-eluting stents; PC-ZES: Phosphorylcholine polymer based zotarolimus-eluting stent; PES: Paclitaxel-eluting stents; SES: Sirolimus-eluting stents; TVR: Target vessel revascularization; BMS: Bare metal stent stents.

ing, held in Barcelona. Meta-analysis of pooled data showed that first-generation DES increased mortality and repeat MI compared to BMS^[34]. High rates of early and late stent thrombosis after discontinuation of dual antiplatelet agents in patients treated with first-generation DES also raised safety concerns^[35,36]. Pathology studies demonstrated that the durable polymers used in first-generation DES could cause a delay in arterial healing, characterized by persistent fibrin deposits, delayed hypersensitivity reactions, and poor endothelialization of the vessel wall, all of which increased the thrombotic risk^[37].

SAFETY AND EFFICACY OF SECOND-GENERATION DES IN STEMI

Second-generation DES were developed to resolve these issues. Stent design and polymeric coating were improved by the use of biocompatible or bioabsorbable polymers. Two RCTs have been published about zotarolimus-eluting stents (ZES) implantation in STEMI patients^[38,39] (Table 2).

The multicenter, prospectively randomized, ZEST-AMI trial included 328 patients who were randomly assigned to ZES (*n* =108), SES (*n* =110), or PES (*n* =110) groups^[38]. Mortality, MI, and ischemia-driven TVR rates at 12 mo were 11.3%, 8.2%, and 8.2%, respectively (*P* = 0.834); there were no differences in mortality, recurrent MI, and ischemia-driven TVR rates. The SES group had 2 acute and 2 subacute cases of stent thrombosis. In the PES group, 3 patients had subacute thrombosis.

The KOMER study was also a multicenter, prospective, single-blind RCT^[39]. The 611 participants were STEMI patients undergoing primary PCI. They were randomized to treatment with ZES (*n* =205), SES (*n* =204), or PES (*n* =202). At 12-mo follow-up, the incidence of cardiac death, MI, or ischemia-driven target lesion revascularization was 5.9% in the ZES group, 3.4% in the SES group, and 5.7% in the PES group, respectively (*P* = 0.457). The rate of stent thrombosis was similar in all 3 groups (approximately 2%).

Two RCTs have studied the use of everolimus-eluting stents (EES) implantation in STEMI patients^[40,42]. The EXAMINATION study was a multicenter, prospective, randomized, all-comer, controlled trial. In this trial, 1498 patients were randomly assigned to receive EES (*n* =751) or BMS (*n* =747)^[40]. At 1-year follow-up, target lesion and vessel revascularization were significantly lower in the EES group (2.1% *vs* 5.0%; *P* = 0.003, and 3.7% *vs* 6.8%; *P* = 0.0077). There were no differences between the EES and BMS groups in all-cause (3.5% *vs* 3.5%, *P* = 1.00) or cardiac death (3.2% *vs* 2.8%, *P* = 0.76) or repeat-MI (1.3% *vs* 2.0%, *P* = 0.32). Stent thrombosis rates differed significantly between EES and BMS groups for both “definite” and “definite or probable” diagnoses (0.5% *vs* 1.9% and 0.9% *vs* 2.5%, respectively; both *P* = 0.019). At the 2-year follow-up, there were significantly fewer target lesion revascularizations in the EES group (2.9% *vs* 5.6% for BMS; *P* = 0.009)^[41]. Composite of all-cause death, any MI, or revascularization did not differ between groups (14.4% *vs* 17.3%, respectively; *P* = 0.11). Definite and probable stent thrombosis rates were significantly lower in the EES group (1.3% *vs* 2.8%; *P* = 0.03).

The XAMI trial randomized 625 patients with acute myocardial infarction (2:1) to receive EES or SES^[42]. Death, nonfatal MI, or any TVR at 1 year was lower at 4.0% for

Table 3 Current polymer-free stents undergoing clinical evaluation

Stent	Study	Platform	Drug	Primary endpoint	Design	Randomized ratio	Stent comparisons (n)	Result
Yukon (Translumina)	ISAR TEST ^[50]	316 L microporous surface	Sirolimus + Probucol	MACE/ST at 1yr	RCT	2:1	Yukon/R-ZES 3002 (2002/1000)	Noninferior
Cre 8 (CID)	NEXT ^[51]	CoCr abluminal reservoirs	Amphilimus	LL at 6 mo	RCT	1:1	Cre 8/PES 323 (162/161)	Superior
BioFreedom DCS (Biosensors)	BioFreedom FIM ^[52]	316 L microstructured surface	Biolimus A9	LL at 12 mo	RCT	1:1:1	Standard dose/low dose Biofreedom/PES 182 (60/62/60)	Noninferior
Vestasync (MIV therapeutics)	VESTASYN II ^[53]	316 L microporous nanofilm Hap	Sirolimus	LL at 4 and 9 mo	RCT	2:1	VESTASync/BMS 75 (50/25)	Superior
Amazonia Pax (Minvasys)	Pax A and Pax B	CoCr nontextured	Paclitaxel	LL at 6 mo	RCT	1:1	PAXA/PES 30 (15/15), PAXB = 100	Noninferior
Yinyi (Liaoning Biomed.Mat)	FREEDOM ^[54]	316 L micropores	Paclitaxel	MACE/ST/TVR	RCT	2:1	Yinyi/SES 1626 (931/449)	Noninferior
Bicare+ (Lepu Medical)	BICARE ^[56]	316 L	Sirolimus + Probucol	TVF at 30 d	FIM	-	$n = 32$	TVF = 9.4%, LL 0.14, ISR = 3.2%
Pronova XR (Vascular Concepts)	EURONOVA XR I ^[55]	Co-Cr	Sirolimus	LL at 6 mo	FIM	-	$n = 50$	In-stent LL 0.45
Focus NP (Envision Scientific)	Nano active FIM	316 L nontextured	Sirolimus nanoparticles	LL at 6 mo	FIM	-	$n = 100$	Ongoing
Mitsu (Meril Medical)	-	CoCr ultrathin struts	Merilimus	-	-	-	Planned	-
Hollow-core DFS (Medtronic)	-	CoCr holes and hollow tube	Sirolimus	-	-	-	Planned	-
Nano+ (Lepu medical)	Nano+	Microporous	Sirolimus	OCT evaluation	FIM	-	$n = 45$	Ongoing

MACE: Major adverse cardiac events; ST: Stent thrombosis; RCT: Randomized control trial; LL: Late lumen loss; R-ZES: Resolute zotarolimus-eluting stents; PES: Paclitaxel-eluting stents; BMS: Bare metal stents; SES: Sirolimus-eluting stents; TVR: Target vessel revascularization; TVF: Target vessel failure; ISR: In-stent restenosis; OCT: Optical coherence tomography; FIM: First-in-man trial.

EES *vs* 7.7% for SES ($P = 0.048$) and 1-year incidence of definite and/or probable stent thrombosis was 1.2% for EES *vs* 2.7% for SES ($P = 0.21$).

The COMFORTABLE AMI is the only RCT by the use of biolimus-eluting stents (BES) in STEMI patients^[43]. A total of 1161 patients were randomized 1:1 to receive BES ($n = 575$) or BMS ($n = 582$). Major adverse cardiac events at 1 year occurred in 24 patients (4.3%) receiving BES and in 49 patients (8.7%) receiving BMS ($P = 0.004$). The difference was driven by a lower risk of target vessel-related repeat MI [3 (0.5%) *vs* 15 (2.7%); $P = 0.01$] and ischemia-driven target-lesion revascularization [9 (1.6%) *vs* 32 (5.7%); $P = 0.001$] in patients receiving BES compared with those receiving BMS. Rates of cardiac death were not significantly different [16 (2.9%) *vs* 20 (3.5%), $P = 0.53$]. Definite stent thrombosis occurred in 5 patients (0.9%) treated with BES and 12 patients (2.1%, $P = 0.10$) treated with BMS.

Recent meta-analyses also showed that EES were associated with significantly lower rates of stent thrombosis than both BMS and PES at 1-year follow-up. In addition, EES were associated with significantly lower rates of cardiac death or MI compared with PES^[44,45].

Pathological analysis also showed that late and very late stent thrombosis occurred less often in the EES (4%) than in the SES (21%; $P = 0.029$) and PES groups (26%; $P = 0.008$). The percentage of uncovered struts was lower in the EES (media $n = 2.6\%$) than in SES (18.0%; $P < 0.0005$) or PES groups (18.7%; $P < 0.0005$). Furthermore, EES was associated with less inflammation, no hypersensitivity, and less fibrin deposit than both SES and PES^[46].

GLIMPSE INTO THE FUTURE: NEXT-GENERATION STENT PLATFORMS FOR STEMI?

A new, self-apposing stent has been developed to reduce malapposition, which may eventually provoke stent thrombosis. In the APPOSITION II study, optical coherence tomography at 3 d after implantation showed a lower rate of malapposed stent struts in the self-apposing BMS group than in the balloon-expandable group (0.58% vs 5.46%, $P = 0.001$)^[47]. In the APPOSITION IV study, patients treated with a self-apposing SES had better apposition ($P = 0.001$) and better coverage at 4-mo follow-up than the balloon-expandable ZES (31.6% vs 3.8%; $P = 0.03$)^[48].

The micronet-mesh-covered stent has been developed to prevent distal embolization. In the MASTER study, complete ST-segment resolution was significantly improved in patients treated with micronet-mesh-covered stent, compared with commercially available BMS or SES (57.8% vs 44.7%; $P = 0.008$)^[49].

NONPOLYMERIC STENTS IN STEMI

Nonpolymeric stents have been developed to avoid polymer-related delayed neointimal healing and late stent thrombosis, and several have undergone clinical investigation (Table 3). However, most of the clinical data have been gathered in low-risk patients without STEMI^[50-56]. A small study showed that polymer-free PES (PF-PES) were noninferior to polymer-based PES (PB-PES) in patients with STEMI, both in terms of target lesion failure (10.9% PB-SES vs 12.0% PF-PES; $P = 0.861$) and definite or probable stent thrombosis (1.8% PB-SES vs 2.0% PF-PES; $P = 1.000$) at one year^[57].

BIORESORBABLE SCAFFOLDS IN STEMI

Fully bioresorbable vascular scaffold (BVS) was developed to overcome problems associated with a durable polymer and metallic scaffold. Disappearance of the stent from the treated site might decrease the risk of stent thrombosis. So far, a few studies with short-term follow-up have been published about bioresorbable vascular scaffold in STEMI or acute coronary syndrome^[58-61]. Further studies in a larger number of patients and long-term follow-up are planned.

The ongoing ISAR-absorb MI trial (A Prospective, Randomized Trial of BVS vs EES in Patients Undergoing Coronary Stenting for Myocardial Infarction, www.clinicaltrial.gov, NCT01942070) tests the clinical performance of the everolimus-eluting BVS vs durable polymer EES in patients undergoing PCI in the setting of acute MI. The primary endpoint is percent diameter stenosis in angiographic follow-up at 6 to 8 mo. Subsequent clinical follow-up will be undertaken up to 5 years.

Another ongoing study is ABSORB STEMI: the TROFI II trial (www.clinicaltrial.gov, NCT01986803), a

prospective, single-blind, noninferiority, European multicenter RCT. The primary endpoint is to assess the neointimal healing score as evaluated by intracoronary optical frequency domain imaging in patients with STEMI and treated with everolimus-eluting BVS at 6 mo follow-up, compared to that of EES. Furthermore, the safety and feasibility of implanting everolimus-eluting BVS in patients with STEMI will be assessed.

CONCLUSION

The second-generation DES significantly reduced TVR compared with BMS, without an increase in mortality, MI, or stent thrombosis rates. In patients with STEMI, the use of second-generation DES appears safer and more efficacious than either BMS or first-generation DES. Results of the ongoing ISAR-absorb trial and ABSORB STEMI: the TROFI II trial will shed light on the potential benefits of the new BVS in the context of STEMI.

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WJC 6th Anniversary Special Issues (5): Myocardial infarction

miRNome in myocardial infarction: Future directions and perspective

Emanuela Boštjančič, Damjan Glavač

Emanuela Boštjančič, Damjan Glavač, Department of Molecular Genetics, Institute of Pathology, Faculty of Medicine, University of Ljubljana, 1000 Ljubljana, Slovenia

Author contributions: Boštjančič E wrote the paper; Glavač D revised the paper critically; Boštjančič E and Glavač D gave the final approval of the version to be published.

Correspondence to: Damjan Glavač, PhD, Professor of Human Genetics, Department of Molecular Genetics, Institute of Pathology, Faculty of Medicine, University of Ljubljana, Korytkova 2, 1000 Ljubljana, Slovenia. damjan.glavac@mf.uni-lj.si
Telephone: +386-1-5437180 Fax: +386-1-5437181

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Abstract

MicroRNAs (miRNAs), which are small and non-coding RNAs, are genome encoded from viruses to humans. They contribute to various developmental, physiological and pathological processes in living organisms. A huge amount of research results revealed that miRNAs regulate these processes also in the heart. miRNAs may have cell-type-specific or tissue-specific expression patterns or may be expressed ubiquitously. Primary studies of miRNA involvement in hypertrophy, heart failure and myocardial infarction analyzed miRNAs that are enriched in or specific for cardiomyocytes; however, growing evidence suggest that other miRNAs, not cardiac or muscle-specific, play a significant role in cardiovascular disease. Abnormal miRNA regulation has been shown to be involved in cardiac diseases, suggesting that miRNAs might affect cardiac structure and function. In this review, we focus on miRNAs that have been found to contribute to the pathogenesis of myocardial infarction (MI) and the response post-MI and characterized as diagnostic, prognostic and therapeutic targets. The majority of these studies were performed using mouse and rat models of MI, with a focus on the

identification of basic cellular and molecular pathways involved in MI and in the response post-MI. Much research has also been performed on animal and human plasma samples from MI individuals to identify miRNAs that are possible prognostic and/or diagnostic targets of MI and other MI-related diseases. A large proportion of research is focused on miRNAs as promising therapeutic targets and biomarkers of drug responses and/or stem cell treatment approaches. However, only a few studies have described miRNA expression in human heart tissue following MI.

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Key words: MicroRNAs; Myocardial infarction; Human; Animal models; Biomarkers and targets

Core tip: MicroRNAs (miRNAs) contribute to various developmental, physiological and pathological processes in the heart. Cardiac diseases show abnormal miRNA regulation. Primary studies of miRNA involvement in cardiac disease analyzed mainly miRNAs that enriched in or specific for cardiomyocytes; however, growing evidence suggests that other cell-type-specific or ubiquitously expressed miRNAs are also involved in cardiovascular disease. miRNAs were found to contribute to the pathogenesis of myocardial infarction (MI) and post-MI. The majority of studies focused on miRNAs in animal models of MI, in human and animal plasma samples of MI (prognostic and diagnostic targets), and on miRNAs as promising therapeutic targets.

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INTRODUCTION

MicroRNAs (miRNAs) are endogenously expressed small non-coding RNA molecules. Genes encoding miRNAs can be found in genomes of almost all organisms, including viruses. Their prime mechanism of action is post-transcriptional repression of gene expression^[1]. It is suggested that the short length (22 nt) maximizes target-gene specificity and minimizes non-specific effects. It is estimated that miRNAs regulate approximately 30% of genes within the human genome^[2]. There are over 2000 miRNAs known to be encoded by human genome. All sequenced and cloned miRNAs from humans as well as from other species are included in database miRBase (v20.0, June 2013, <http://www.mirbase.org>)^[3].

Mechanism of action of miRNAs

Biogenesis (including genes encoding miRNAs), transcription and processing are beyond the scope of this review and are described elsewhere^[1,2,4,5]. As mentioned above, miRNAs prime mechanism of action is repression of gene expression. By sequence-specific binding to the 3'-untranslated region (3'-UTR) of mRNAs, miRNA affects stability of the transcripts and cause mRNA degradation, which is the main mechanism in plants and happens when complementarity between miRNA and mRNA is perfect, or cause protein synthesis repression (translational repression), which happens when base pairing between these two molecules is incomplete and is the canonical mechanism in animals^[1,2,4,5]. Due to incomplete base pairing in animals and humans, each miRNA could influence translation of many different mRNAs without degrading it (*i.e.*, over 200 predicted target genes) and vice versa each mRNA may be influenced by different miRNA. It appears that the most efficient translational inhibition is provided through the multiplicity, which is the consequence of numerous target sites for the same miRNAs within 3'-UTR of the same mRNA (cooperative action of multiple identical miRNAs), and through cooperativity, which is due to numerous target sites for the different miRNAs within 3'-UTR of the same mRNA. miRNA access to the UTRs could be on one hand restricted by proteins or mRNA secondary structures, and on the other hand these structures and protein binding may facilitate recognition of the mRNA targets^[6]. Some miRNAs might also have other functions, although translational repression has been suggested to be the canonical one^[2,4].

miRNA in regulating physiological functions

Different approaches in *in vitro* and *in vivo* experiments have been used to reveal function of majority of miRNA. Using mutated miRNA or its mutated complementary site within mRNA, consequently disrupting regulation of mRNA by miRNA, leads to the determination of the phenotypic consequence of this non-binding. Another possibility is use of transgenic constructs of either 3'-UTR or miRNA expressing vector and ectopic expres-

sion of the either miRNA or mRNA^[1,7]. Perhaps the best evidence that miRNA are playing a significant role in normal physiological functions was established, when the components of the miRNA biogenesis pathway were depleted^[8]. In normal cell conditions, miRNAs can repress translation in different ways: (1) as a switching-off the targets, when protein production is reduced to inconsequential levels in a cell type, where target mRNAs should not be expressed; (2) as fine-tuning expression of target gene, when protein output can be adjusted in a way, which provides customized expression in one cell type and uniformly expressed level within another cell type; and (3) as neutralizers of target gene expression, when mRNA downregulation by miRNAs is negated through feedback processes^[1]. The role of miRNAs can be combinatorial (defined as cooperativity), different in different cell types, and either specific or housekeeping^[9].

Through the studies of expression profiling of normal and disease tissues it has been shown that miRNAs are expressed in spatial as well as in temporal manner. *miR-208* is a good example of expression in tissue-specific manner. Its expression can be detected specifically in the hearts, as well can be *miR-122* found primarily in the liver. As an example of cell-type-specific miRNAs are *miR-223*, which is primarily expressed in granulocytes, and *miR-1* and *miR-133*, which are believed to be myocyte-specific^[10]. miRNAs are involved in a myriad of biological processes, including proliferation, apoptosis, metabolism, differentiation, epithelial-to-mesenchymal transition, regulation of insulin secretion, division of stem cells, embryonic development and patterning, fetal growth, immune system, including resistance to viral infection and vice versa viral production (in a case of HCV), *etc.*^[8]. miRNA activity is believed to have crucial role in regulatory role in maintaining tissue identity during embryogenesis as well as in adult life. Distinct miRNA expression profile, with completely different gene expression patterns might be observed in every cell type at each developmental stage^[9-11].

Target prediction and bioinformatics

As mentioned above, miRBase is major database of all known miRNAs, which can also predict possible miRNA targets^[3]. Predicting possible miRNA binding sites for specific mRNAs or potential targets for certain miRNA is usually the first step in target identification and for this purpose numerous computational methods have been developed. Main characteristics that are included in established programs are: evolutionarily conservation of the complementary 3'-UTR sequence, quality and stability of mRNA:miRNA pairing and involvement of "seed sequence". It is believed that for base pairing the most important is "seed sequence" of the miRNA (2-8 nt at the 5'-end) and its interaction with seven consecutive nucleotides in the target mRNA^[12]. However, all predicted targets have to be validated *in vitro* and *in vivo* since none of these programs can independently validate the targets^[7,13]. Due the facts that 3'-UTR sites with

perfect complementarity to the miRNA are not necessary functional and that mRNA sites with imperfect complementarity can themselves be very good miRNA targets, are bioinformatic analysis more prone to false positives^[6]. Therefore, experimental demonstration that overexpression of the miRNA represses a luciferase reporter fused to the 3'-UTR of the predicted target and that this repression is not established by point mutations in the 3'-UTR target sequence is the gold standard for miRNA target identification^[13,14]. Finally, association of mRNA:miRNA pairs with disease pathogenesis should be confirmed by expression profiling in human diseases by co-expression analyses^[7,14]. Human MicroRNA Disease Database identifies all disease-related miRNAs with their tissue expression patterns^[15]. Further, Tarbase lists experimentally validated miRNA targets for all organisms^[12].

miRNAs AND DISEASE

Mutations, single-nucleotide polymorphisms and the epigenetics of miRNAs

There are several genetic and epigenetic abnormalities within miRNA genes that might contribute to a wide range of diseases. These abnormalities include small- and large-scale genomic alterations, as are rearrangements and chromosomal translocations, copy-number variation, nucleotide expansion, and single-nucleotide polymorphisms (SNPs) that beside protein-coding region also affect regions that code for non-coding RNAs. First, it has been shown that approximately 50% of the miRNA genes are encoded within fragile chromosomal sites or sites that are prone to cancer-associated rearrangements^[10]. Second, although some SNPs are silent and cause no obvious functional consequence, other might cause disruption of binding between miRNAs and their targets, which can potentially lead to gain or loss of the function of miRNA or its target gene and consequently contribute to the disease state^[16]. Variants identified in miRNA or their precursors (pri-miRNA or pre-miRNA) that beside targeting might also affect the processing and expression of miRNAs, are rarely observed. However, potential of variation in miRNA target sites is more huge^[6,16]. Third, the aberrant DNA methylation of gene promoters has been shown to result in the inactivation of different genes, including miRNAs, and in parallel, miRNAs can also regulate proteins involved in DNA methylation^[17].

Aberrant expression of miRNAs

Epigenetic mechanisms and genomic abnormalities frequently lead to abnormal miRNA expression profiles thus causing pathogenetic events in diseases. Numerous advances in miRNA research and numerous expressions profiling of diseased human tissue are suggesting that miRNAs are associated with various pathological conditions. miRNAs have been linked to wide range of diseases, including cancer genetic and immunological disorders, neurodegenerative and cardiovascular disorders^[10].

THERAPEUTIC POTENTIAL OF miRNAs

miRNA expression patterns are dynamically regulated during various diseases and can also be used for pharmacological manipulation. Studies have demonstrated that the systemic use of antagomirs is well suited to block miRNA function in small animal models. For targeting a specific miRNA or disrupting binding between miRNA and its target mRNA the chemically modified oligonucleotides have been developed. miRNAs as small molecules of approximately 22 nt in length are more feasible delivered *in vivo*. Synthetic miRNAs can be therefore delivered systematically and may thus serve as therapeutic targets in the future^[18].

Replenishing small RNAs

Underexpressed miRNA might be restored by reintroduction of the mature miRNA into the target tissue consequently restoring regulation of the miRNA target gene. miRNAs as potential therapeutic agents can be easily targeted and delivered to the appropriate tissue. Three major approaches are described below. Artificial miRNA or miRNA “mimics” enhance the expression of beneficial miRNAs. Artificial miRNAs are transient transfections of double-stranded miRNAs and possess the ability to bind to the homologous target site in various mRNAs. Another option is the introduction of a viral vector or plasmid expressing a specific miRNA from a short hairpin (sh) duplex (pre-miRNA-like shRNA). A high level of shRNA might lead to effective target knockdown; however, it may also saturate the miRNA biogenesis pathway and lead to off-target effects with fatal consequences. Therefore, another possibility arises, namely miRNA scaffolds. In scaffolds of endogenous pri-miRNA or pre-mRNA, siRNA is inserted and introduced to the target tissue leading to the degradation of homologous mRNA. This approach is advantageous in terms of specificity and stability over conventional shRNA because both siRNA and shRNA may trigger a non-specific interferon response in addition to off-target effects. As an example in cardiovascular disease, overexpression of *miR-133* was used in a study of cardiac hypertrophy. By adenovirus delivery of a miRNA expression cassette, expression of *miR-133* was restored, which results in protection of experimental animals from agonist-induced cardiac hypertrophy^[18].

Inhibiting small RNAs

ASOs are short single-stranded antisense oligonucleotides, which are called anti-miRNA oligonucleotides, AMOs or antagomirs when talking about inhibition of miRNA. Antagomirs have been shown to efficiently and specifically silence endogenous miRNAs in mice. Overexpressed miRNA can also be downregulated by reducing the loop region of the miRNA precursor (pre-miRNA). The loop regions of different pre-miRNAs are not conserved and might therefore limit their application. Another approach is to use miRNA sponges, which are miRNA inhibitory transgenes containing multiple tandem binding

sites for an endogenous miRNA and can inhibit several closely related miRNAs. miRNA sponges may be useful for sequestering a miRNA family with overlapping and redundant targets. miR-masks and miR-erasers have also been developed. Similarly to a miR-sponge, a miR-eraser sequesters more than one miRNA, except that there are only two copies of the antisense sequence. For masking the miRNA binding site on the target gene, miR-mask or miRNA masking antisense approach has been designed, which forms a duplex with target mRNA. They are also called antisense oligodeoxynucleotides (ODNs). Two approaches have been used in the context of studying cardiovascular diseases. A miRNA decoy using miRNA sponges was designed and used in research studying the effect of *miR-133* in the pathogenesis of cardiac hypertrophy. Another approach used ODNs entirely complementary to the miRNA target motifs in the 3'-UTR of 2 cardiac pacemaker channel genes, *HCN2* and *HCN4*^[18,19].

Delivering miRNAs or its inhibitor to the target tissue

Current limitations exist in the following areas and need improvement: the efficiency of delivery of miRNA therapeutics to target tissues; systemic administration of drug; the potential inhibition of non-target genes (off-target effects); redundancy in the efficacy of different miRNAs; potential toxicity; and immunogenic responses. Modifications have improved the stability of miRNAs or blocked their inhibition (*i.e.*, nuclease resistance and pharmacokinetic properties such as half-life in serum and cellular uptake). Stabilization and facilitation of intravenous delivery of antagomirs could be improved by chemical modifications and cholesterol conjugations. However, toxicity due to chemical modifications should be taken into account. Local administration in easily accessible tissues has been used in the majority of the developed protocols; a major challenge remains for tissue- and cell-type-specific targeting. Viral and non-viral delivery systems have been developed in conjugation with homing signals for tissue- or cell-type-specific delivery, *e.g.*, linked to lipids and/or proteins, cationic liposomes, cholesterol, bacterial phage, aptamers, *etc.*^[18,19].

miRNA IN MYOCARDIAL INFARCTION

MicroRNA in cardiovascular diseases

miRNAs contribute to the regulation of developmental, as well as physiological and pathological processes in the heart. Loss of cardiomyocyte renewal is a hallmark of numerous cardiac diseases, which might also influence miRNA expression patterns in the diseased heart^[20]. Primary studies of miRNA involvement in hypertrophy, heart failure and myocardial infarction analyzed miRNAs that are enriched in or are specific for cardiomyocytes; however, growing evidence suggest that other miRNAs, not cardiac- or muscle-specific, play a significant role in cardiovascular disease^[21-24]. Using experimental animals and human samples dysregulation of specific miRNAs has been shown that are distinct than those involved in

other heart diseases as are hypertrophy and heart failure (HF). Cell lines and animal models of different forms of myocardial ischemia, including myocardial infarction (MI), have been used to perform miRNA microarray expression profiling^[20]. It has been shown that in response to limited amount of oxygen, numerous miRNAs are up- or downregulated. Many of dysregulated miRNAs are dependent on a transcription factor that plays an important role in response to low oxygen, hypoxia-inducible-factor. Further analyses showed that oxidative stress also activates other transcription factors that beside miRNA expression influence different homeostatic and physiological processes as are metabolism, angiogenesis, cell survival and oxygen delivery^[25,26]. Numerous other pathways are activated in response to MI, including apoptosis and fibrosis, as well as numerous cell types such as cardiomyocytes, immune cells, fibroblasts and endothelial cells (ECs)^[27]. However, the majority of studies were performed in terms of expression analysis and target gene identification, with only one publication focusing on MI, specifically, target site polymorphisms and the risk for MI.

Cardiac and muscle specific miRNAs in heart

There are five miRNAs recognized as muscle- and/or cardiac-specific or enriched, *miR-1*, *miR-133*, *miR-206*, *miR-208* and *miR-499*. For *miR-1* and *miR-133* it is believed that are muscle-specific and that regulate heart development^[28]. *miR-208* has been identified as cardiac-specific and *miR-499* as cardiac-enriched.

In the current review we have focused on miRNAs involved in MI pathogenesis and their diagnostic, prognostic and therapeutic potential regarding MI. The function of various miRNAs analyzed in cell lines, animal models of MI and patients with MI are presented. Table 1 summarizes all free-circulating miRNAs in experimental model of MI and human MI, describes their suggested function and predicted targets. We further overviewed the results of free-circulating miRNAs in different bodily fluids of patients and/or an animal model with MI. Table 2 summarizes all miRNAs as potential diagnostic and/or prognostic targets in MI. Lastly, therapeutic opportunities using miRNA strategies in the context of MI are also presented. More detailed description of all these miRNAs is below.

Cell line models

miR-21: *miR-21* was upregulated after inducing injury of cardiac myocytes using H₂O₂, and H₂O₂-induced cardiac cell death and apoptosis were increased by a *miR-21* inhibitor. Programmed cell death 4 (PDCD4) has been identified as a target of *miR-21*, and activator protein 1 (AP-1) has been identified as a downstream signaling molecule of PDCD4^[29]. All miRNAs with suggested role in apoptosis in MI are summarized in Figure 1.

miR-15b and miR-106b: After retrieving 119 MI-related miRNAs from publications, GO and pathway analyses

Table 1 miRNAs with suggested role in experimental models of myocardial infarction and in myocardial infarction in humans

miRNA	Role/function	Expression in MI	Target genes	Species	Ref.
<i>miR-1</i>	After heat-shock up, protective against I/R	Nd	Repressing pro-apoptotic and up-regulating anti-apoptotic genes	Mouse	[40]
	Nd	Down	Nd	Mouse	[59]
	Pro-apoptotic	Up	IGF1	Rat	[45]
	Pro-arrhythmogenic	Up	Ion channels: Cx43, Kir2.1	Rat	[46]
	Predictive	Down, up	Predictive	Human	[55,56]
<i>miR-15b</i>	Anti-angiogenic	Down	Suggested VEGF and Ang2	Endothelial cells	[30]
<i>miR-21</i>	H ₂ O ₂ induced cell injury	Up	PDCD4	Cardiomyocytes	[29]
	After heat-shock up, protective against I/R	Nd	Repressing pro-apoptotic and up-regulating anti-apoptotic genes	Mouse	[40]
	Response to I/R	Up in cardiac fibroblasts	PTEN	Mouse	[23]
	Anti-apoptotic	Down, up	PDCD4	Rat	[47]
	Pro-arrhythmogenic	Up	Sprouty-1, collagen I, collagen III	Rat	[48]
<i>miR-24</i>	Anti-fibrotic	Down	Furin	Mouse	[35]
	Anti-angiogenic, induce endothelial cell apoptosis	Down in cardiomyocytes and fibroblasts, up in endothelial cells	GATA2, PAK4 eNOS	Mouse Mouse	[36] [37]
	After heat-shock up, protective against I/R	Nd	Repressing pro-apoptotic and up-regulating anti-apoptotic genes	Mouse	[40]
<i>miR-29</i> family	Anti-fibrotic	Down	Proteins involved in fibrosis (COL1A1-2, COL3A1, FBN1, ELN)	Mouse, human	[34]
<i>miR-29b</i>	Anti-fibrotic	Down	Proteins involved in fibrosis (COL1A1, COL3A1, α SMA)	Rat	[52]
<i>miR-34a</i>	Pro-apoptotic	Up	ALDH2	Rat	[49]
<i>miR-92a</i>	Anti-angiogenic	Up	ITGA5	Mouse	[38]
<i>miR-101a/b</i>	Anti-fibrotic	Down	c-Fos	Rat	[50]
<i>miR-106b</i>	Anti-apoptotic	Up	p21	Cardiomyocytes	[30]
<i>miR-133a</i>	Nd	Down	Nd	Mouse	[59]
	Predictive	Down	Predictive	Human	[55,56]
<i>miR-133b</i>	Predictive	Down	Predictive	Human	[55,56]
<i>miR-146a</i>	Predictive: inflammation and VR	Up	Predictive	Human	[57]
<i>miR-150</i>	Predictive: inflammation and VR	Down	Predictive	Human	[57]
<i>miR-155</i>	Predictive: inflammation and VR	Down	Predictive	Human	[57]
<i>miR-206</i>	Pro-apoptotic	Up	IGF1	Rat	[45]
<i>miR-208a</i>	Nd	Down	Nd	Mouse	[59]
	Predictive	Up	Predictive	Human	[55]
<i>miR-320</i>	Pro-apoptotic	Down	HSP20	Mouse	[41]
<i>miR-494</i>	Activation of Akt pathway	Down	Pro- and anti-apoptotic proteins (PTEN, ROCK1, CaMKII; FGFR2, LIF)	Mouse	[42]
<i>miR-499</i>	Nd	Down	Nd	Mouse	[59]
<i>miR-711</i>	Involved in anti-fibrotic effect of pioglitazone	Down	SP1	Rat	[51]
<i>miR-874</i>	Regulated by Foxo3a in necrosis	Up	Caspase 8	Mouse	[39]

I/R: Ischemia/reperfusion; MI: Myocardial infarction; Nd: Not determinate; VR: Ventricular rupture.

for their predicted gene targets demonstrated that these dysregulated miRNAs were enriched in cardiovascular-related phenotypes. By highlighting miRNA-gene networks, overall relationships between miRNAs and gene targets were discovered, particularly in apoptosis and angiogenesis. Experimental data identified *miR-106b* as an anti-apoptotic modulator through inhibition of p21 expression and *miR-15b* as an anti-angiogenic miRNA with the possible targets vascular endothelial growth factor and Ang2 (angiopoietin 2)^[30]. All miRNAs with suggested role in angiogenesis in MI are summarized in Figure 2.

To investigate the possible release of miRNAs from activated platelets, the miRNA content of platelets was screened from control patients and patients with MI. Nine miRNAs found to be differentially expressed in MI patients compared with healthy controls were screened,

and 8 of these were decreased in MI patients. Of these, *miR-22*, *miR-185*, *miR-320b* and *miR-423-5p* increased after aggregation in the supernatant of platelets and were depleted in thrombi aspirated from MI patients. Platelets from patients with MI exhibit a loss of specific miRNAs, and activated platelets shed miRNAs that can regulate EC gene expression^[31].

Mouse models

Whole genome microarray analysis: Genome-wide mRNA and miRNA expression profiles were performed at three time points post-MI: 2 d, 2 wk and 2 mo. The majority of differentially expressed miRNAs were uniquely regulated at each of the time points analyzed. Bioinformatic analysis demonstrated that several genes and miRNAs in various pathways are regulated in a tem-

Table 2 miRNAs as potential diagnostic and prognostic biomarkers in myocardial infarction

miRNAs as potential biomarker	Role of biomarker	Expression in body fluid	Species and body fluid	Ref.
<i>let-7b</i>	Potential diagnostic value	Up	Plasma; human	[72]
<i>let-7f</i>	Differentiating TTC and MI	Down	Plasma; human	[79]
<i>miR-1</i>	Detection of AMI and AP	Up	Exosome, serum; human and mouse	[59]
	Correlation with MI size	Up	Serum; rat and human	[60]
	Differentiating AMI and AP	Up	Serum; human	[61]
	Differentiating AMI and other cardiovascular diseases	Up	Plasma; rat and human	[67]
	Similar time course to cTnI and the same trend to cTnI concentration	Up	Plasma and tissue; human and mouse	[68,70]
	Differentiating AMI and non-AMI	Up	Plasma; human	[69]
	AMI biomarkers, not superior to cTnT	Up	Plasma; human	[71]
	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Up	Plasma; human	[76]
	No association with 30 d mortality post-MI and diagnosis of HF	Up	Plasma; human	[81]
	Biomarker for AMI, correlated with renal elimination	Up	Plasma, urine; human, pig	[85]
	Detected in urine	Up	Urine; rat	[86]
<i>miR-16</i>	Differentiating TTC and MI	Down	Plasma; human	[79]
	Higher risk of impaired LV contractility	Up	Plasma; human	[83]
<i>miR-21</i>	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Nd	Plasma; human	[76]
	Differentiating NSTEMI and CHF	Up	Plasma; human	[78]
	Time-dependent changes 2-90 d post-MI	Down, up	Plasma; human	[80]
<i>miR-26a</i>	Differentiating TTC and MI	Down	Plasma; human	[79]
<i>miR-27a</i>	High risk of impaired LV contractility	Up	Plasma; human	[83]
<i>miR-29a</i>	Time-dependent changes 2-90 d post MI	Up	Plasma; human	[80]
<i>miR-29b</i>	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Nd	Plasma; human	[76]
<i>miR-30a</i>	Potential diagnostic value	Up	Plasma; human	[72]
<i>miR-30c</i>	Correlation with MI size	Up	Whole blood; human	[64]
<i>miR-34a</i>	Prognostic: correlated with LV end diastolic dimension	Up	Exosomes, serum; human	[62]
<i>miR-101</i>	Higher risk of impaired LV contractility	Down	Plasma; human	[83]
<i>miR-126</i>	The same trend to cTnI expression	Down	Plasma; human	[70]
	Positive association to the risk for MI	Nd	Plasma; human	[84]
<i>miR-133a</i>	Detection of AMI, AP: biomarker for cardiomyocyte death	Up	Exosome, serum; human and mouse	[59]
	AMI biomarker, correlation to cTnI	Up	Plasma and whole blood; human	[66]
	Differentiating AMI and other cardiovascular diseases	Up	Plasma; rat and human	[67]
	Similar time-course to cTnI	Up	Plasma and tissue; human and mouse	[68]
	AMI biomarkers, not superior to cTnT	Up	Plasma; human	[71]
	Differentiating AMI and AP, positive correlation to severity of coronary stenosis	Up	Plasma; human	[75]
	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Up	Plasma; human	[76]
	Differentiating TCC and MI	Up	Plasma; human	[79]
	Biomarker for AMI, correlated with renal elimination	Up	Plasma, urine; human, pig	[85]
<i>miR-133b</i>	Similar time-course to cTnI	Up	Plasma and tissue; human and mouse	[68]
<i>miR-134</i>	Differentiating AMI and AP	Up	Serum; human	[61]
<i>miR-145</i>	Correlation with MI size	Up	Whole blood; human	[64]
<i>miR-146a</i>	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Nd	Plasma; human	[76]
<i>miR-150</i>	Associated with LV remodeling	Down	Plasma; human	[82]
	Higher risk of impaired LV contractility	Down	Plasma; human	[83]
<i>miR-155</i>	Prognostic for cardiac death within 1 yr after MI	Up	Serum; human	[63]
	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Nd	Plasma; human	[76]
<i>miR-181c*</i>	Novel mirna dysregulated during MI	Nd	Whole blood; human	[65]
<i>miR-186</i>	Differentiating AMI and AP	Up	Serum; human	[61]

<i>miR-192</i>	Prognostic for development of ischemic HF	Up	Exosomes, serum; human	[62]
<i>miR-194</i>	Prognostic: correlated with LV end diastolic dimension	Up	Exosomes, serum; human	[62]
<i>miR-195</i>	Potential diagnostic value	Up	Plasma; human	[72]
<i>miR-197</i>	Negative association to the risk for MI	Nd	Plasma; human	[84]
<i>miR-208</i>	Differentiating AMI and AP	Up in AP compared to AMI	Serum; human	[61]
	Differentiating AMI and other cardiovascular diseases	Up	Plasma; rat and human	[67]
	Time-dependent changes 2-90 d post MI	Up	Plasma; human	[80]
	Detected in urine	Up	Urine; rat	[86]
<i>miR-208b</i>	AMI biomarkers, correlation to cTnT but not superior to cTnT	Up	Plasma; human	[71,76]
	Differentiating STEMI and NSTEMI	Higher in STEMI	Plasma; human	[73]
	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Up	Plasma; human	[76]
	Higher risk for 30 d mortality post-MI and HF	Up	Plasma; human	[81]
	Biomarker for AMI, correlated with troponin	Up	Plasma, urine; human, pig	[85]
<i>miR-223</i>	Differentiating AMI and AP	Up	Serum; human	[61]
	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Down	Plasma; human	[76]
	Negative association to the risk for MI	Nd	Plasma; human	[84]
<i>miR-328</i>	AMI biomarker, correlation to cTnI	Up	Plasma and whole blood; human	[66]
<i>miR-380*</i>	Prognostic for cardiac death within 1 yr after MI	Up	Serum; human	[63]
<i>miR-423-5p</i>	Before PCI compared to after	Up	Plasma; human	[77]
<i>miR-499</i>	Differentiating AMI and AP	Up in AP compared to AMI	Serum; human	[61]
	Differentiating AMI and other cardiovascular diseases	Up	Plasma; rat and human	[67]
	Similar time course to cTnI	Up	Plasma and tissue; human and mouse	[68]
	AMI biomarkers, correlation to cTnT but not superior to cTnT	Up	Plasma; human	[71,76]
	Differentiating STEMI and NSTEMI	Higher in STEMI	Plasma; human	[73]
	Differentiating MI, CHF and unstable AP	Up	Plasma; human	[74]
	Associated with various degree of cardiovascular damage (AMI, viral myocarditis, diastolic dysfunction, acute HF)	Up and also in acute HF	Plasma; human	[76]
	Differentiating NSTEMI and CHF	Up	Plasma; human	[78]
	Higher risk for 30 d mortality post MI and HF	Up	Plasma; human	[81]
	Biomarker for AMI, correlated with renal elimination	Up	Plasma, urine; human, pig	[85]
<i>miR-1915</i>	Novel miRNA dysregulated during MI	Nd	Whole blood; human	[65]
11 miRNAs	Prognosis after MI	Up and down	Serum; human	[63]
20 miRNAs	Predicting AMI (96% specificity; 90% sensitivity; 93% accuracy)	Up and down	Whole blood; human	[64]
A subset of miRNAs	Dysregulated during AMI course	Nd	Whole blood; human	[65]
34 miRNAs	AMI biomarkers	20 up, 14 down	Plasma and tissue; human and mouse	[68]
19 candidate miRNAs	Prediction for risk of MI	Nd	Plasma; human	[84]

AMI: Acute myocardial infarction; AP: Angina pectoris; cTnI: Cardiac troponin I; CHF: Chronic heart failure; cTnT: Cardiac troponin T; HF: Heart failure; LV: Left ventricle; MI: Myocardial infarction; Nd: Not determine; NSTEMI: Non-ST-elevation MI; PCI: Percutaneous coronary intervention; STEMI: ST-elevation MI; TTC: Takotsubo cardiomyopathy.

poral or phenotype-specific manner^[32]. In another study, a mouse MI was induced and one week after MI, a set of 29 upregulated miRNAs was found in the left ventricle originating from the *Dlk1*-deiodinase type 3 gene (*Dio3*) genomic imprinted region, which has been identified as a hallmark of pluripotency and proliferation. This miRNA signature was associated with an increase in expression of the *Dio3* located in this region. *Dio3* is a fetally expressed enzyme associated with cell proliferation, which was shown to be upregulated in cardiomyocytes. These data suggest that a regenerative process is initiated, but not completed, in adult cardiomyocytes after MI^[33].

***miR-29* and fibrosis:** One of the first studies regarding MI was comparing expression profiles of miRNA from mouse border zone of the infarcted region as well as from the remote myocardium 3 and 14 d after MI. The *miR-29* family was downregulated in the region of the heart adjacent to the infarct. It has been shown that downregulation of *miR-29b* with anti-miRs induces the expression of collagen and that overexpression of *miR-29* reduces collagen. Three days after the MI, in the infarcted region, *miR-29* downregulation correlated with upregulation of collagen types I and III (COL1A1, COL1A2, COL3A1) and fibrillin, and in the remote myo-

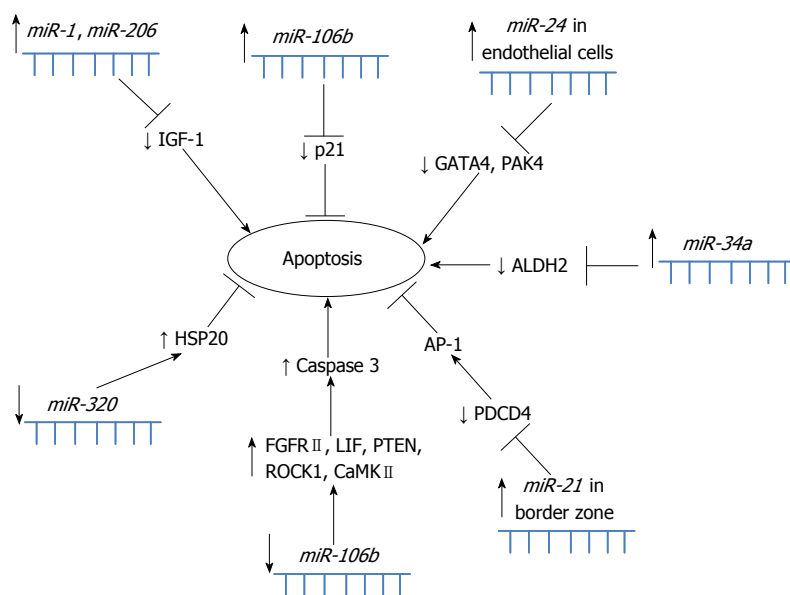


Figure 1 Schematic overview of miRNAs involved in apoptosis in myocardial infarction.

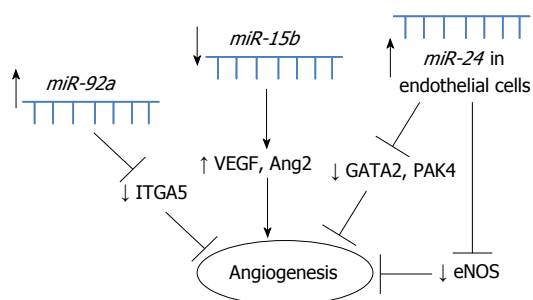
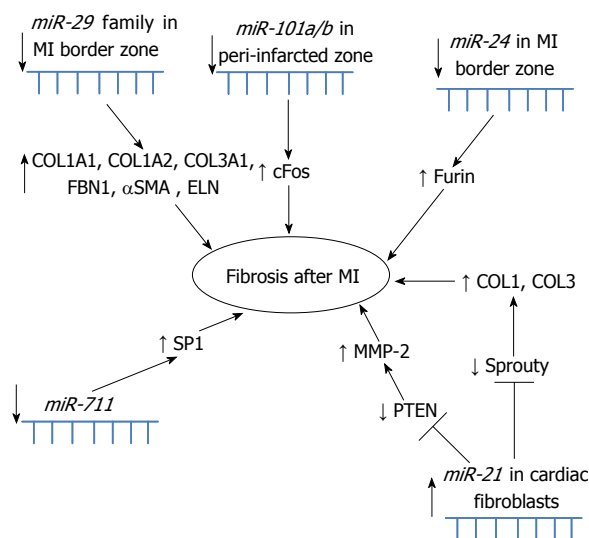


Figure 3 Schematic overview of miRNAs involved in fibrosis after myocardial infarction.



cardium expression of elastin was increased. The *miR-29* family was thus identified as a regulator of fibrosis^[34]. All miRNAs with suggested role in fibrosis in MI are summarized in Figure 3.

***miR-24*, fibrosis and angiogenesis:** The downregulation of *miR-24* in a mouse MI model was closely related to extracellular matrix remodeling. Intra-myocardial injection of *miR-24* was able to improve heart function and attenuate fibrosis in the infarct border zone. *In vitro* experiments suggested that the upregulation of *miR-24* could reduce fibrosis and decrease the differentiation and migration of cardiac fibroblasts (CFs). Transforming growth factor β (TGF- β) increased *miR-24* expression, and overexpression of *miR-24* reduced TGF- β secretion and Smad2/3 phosphorylation in CFs. Furin was found to be a potential target for *miR-24* in fibrosis and both protein and mRNA levels of furin were regulated by *miR-24* in CFs^[35]. *miR-24* is markedly upregulated after cardiac ischemia and it has been also shown to be enriched in cardiac ECs. *miR-24* has been reported to induce apoptosis in ECs and abolishes endothelial capillary network formation by targeting the endothelium-

enriched transcription factor GATA2 and the p21-activated kinase PAK4. MI size in mice has been limited by blocking endothelial *miR-24*. Reduced MI size as well as preserved cardiac function and survival were probably due to prevention of endothelial apoptosis and enhancement of vascularity as a consequence of blocked *miR-24*^[36]. Another mouse model showed that after a MI induction, *miR-24* expression was lower in the peri-infarct tissue and its resident cardiomyocytes and fibroblasts, while it increased in ECs. Local adenovirus-mediated *miR-24* decoy delivery increased angiogenesis and blood perfusion in the peri-infarct myocardium, reduced infarct size, induced fibroblast apoptosis and overall improved cardiac function. The *miR-24* decoy increased apoptosis in cardiomyocytes. *In vitro* *miR-24* inhibition enhanced EC survival and proliferation and induced cardiomyocyte and

fibroblast apoptosis. Endothelial nitric oxide synthase has been identified as a novel direct target of *miR-24* in human cultured ECs and *in vivo*^[37].

***miR-92a* and angiogenesis:** *miR-92a* has been shown to control the growth of new blood vessels (angiogenesis). Systemic administration of antagomir-92a led to enhanced blood vessel growth and functional recovery of damaged tissue. Overexpression of *miR-92a* blocked angiogenesis and vessel formation. *miR-92a* was shown to be upregulated after induction of acute MI (AMI). Antagomir-92a treatment reduced the infarct size, suppressed the number of apoptotic cells and augmented the number of *in vivo* perfused vessels in the infarct border zone. Among its targets are several pro-angiogenic proteins, including integrin subunit $\alpha 5$ ^[38].

***miR-874* and necrosis:** Another study revealed that in response to H₂O₂ treatment, *miR-874* was substantially increased. Knockdown of *miR-874* attenuated necrosis in the cellular model and also MI in the mouse model. As downstream mediator and target of *miR-874* was identified caspase-8. Caspase-8 was able to antagonize necrosis. When suppressed by *miR-874*, caspase-8 lost the ability to repress the necrotic program. Foxo3a was identified as a transcriptional repressor of *miR-874* expression. This study determined a novel myocardial necrotic regulatory model consisting of Foxo3a, *miR-874* and caspase-8^[39].

***miR-1*, *miR-21*, *miR-24*, *miR-320* and ischemia-reperfusion:** Heat-shock treatment protects the heart against ischemia-reperfusion (I/R) injury. A significant induction and increase of *miR-1*, *miR-21* and *miR-24* has been observed in hearts of mice, which were subject to cytoprotective heat-shock (HS). miRNAs isolation from HS mice and injection into non-HS mice, resulted in significantly reduction of the infarct size in the heart following global I/R injury. Further analysis showed that reduction in MI size is accompanied by downregulation of expression of genes that induce apoptosis and upregulation of those that reduce apoptosis. These results showed that in the non-heat-shocked mice, miRNA function in heat-shock-like protection against I/R. Proposed mechanism of miRNAs action is through repression of pro-apoptotic genes (caspases 1, 2, 8, and 14, Bid, Bcl-10, Cidea, Ltbr, Trp53, and Fas) and induction of anti-apoptotic genes (Bag-3 and Prdx2). Through administration of *miR-21*, it has been shown that chemically synthesized miRNA can reduce MI size, an outcome that was blocked with a *miR-21* inhibitor^[40]. Another miRNA in the mouse hearts with I/R has been shown to be dysregulated, *miR-320*. *miR-320* was shown to be significantly decreased after MI and to target heat-shock protein 20. Experiments involving cardiac-specific overexpression of *miR-320* in transgenic mice resulted in increased apoptosis and infarct size in the hearts with I/R, and treatment with antagomir-320 reduced the infarct size^[41].

***miR-21*, I/R and fibrosis:** Further research on I/R

models led to the identification of miRNAs with significant expression changes on days 2 and 7 post-I/R. Elevated *miR-21* levels were observed on day 2 as well as on day 7; however, *miR-21* induction in response to I/R was limited to CFs. CFs were shown to be the major cell type in the infarct zone. A marked decrease in phosphatase and tensin homolog (PTEN), a target of *miR-21*, has also been observed in the infarct zone. This decrease has been associated with increased matrix metalloproteinase-2 (MMP-2) expression, suggesting a *miR-21*-PTEN-Akt-MMP-2 pathway in CFs after MI^[23].

***miR-494* and apoptosis in I/R:** A mouse model with cardiac-specific *miR-494* overexpression showed improved recovery of contractile performance during the reperfusion period. This was accompanied by a reduction of apoptosis in transgenic mice and reduced MI in I/R. Cultured adult cardiomyocytes with short-term overexpression of *miR-494* showed an inhibition of caspase-3 activity and reduced cell death after stimulated I/R. *miR-494* inhibited three pro-apoptotic (PTEN, ROCK1, CaMK II) as well as two anti-apoptotic proteins (FGFR2 and LIF). *miR-494* targets both pro- and anti-apoptotic proteins and was downregulated in human infarcted hearts. Divergent targets of a miRNA may work unequally to balance a common signaling pathway and eventually affect its functional consequences^[42].

Rat models

Microarray analysis: Using genome-wide expression profiling of miRNAs in an ischemic myocardium from rat, seventeen miRNAs were shown to be significantly dysregulated during the AMI progression. Expression was analyzed 2, 7 and 14 d after AMI. On day 2, four miRNAs were upregulated (*miR-31*, *miR-223*, *miR-18a* and *miR-18b*) and two were downregulated (*miR-451* and *miR-499-5p*). On day 7, four miRNAs were upregulated (*miR-31*, *miR-214*, *miR-199a-5p* and *miR-199a-3p*) and seven were downregulated (*miR-181c*, *miR-181d*, *miR-499-5p*, *miR-29b*, *miR-26b*, *miR-126* and *miR-1*). On day 14, five miRNAs were upregulated (*miR-214*, *miR-923*, *miR-711*, *miR-199a-3p* and *miR-31*). Some of these dysregulated miRNAs were related to processes included in response to low oxygen as are hypoxia, inflammation, and fibrosis^[43]. In another study, propranolol was chronically administered to induce reversal of the MI. A long-term MI model in rats was established and microarray data analysis showed that long-term propranolol administration resulted in 18 of 31 dysregulated miRNAs undergoing reversed expression. *miR-1*, *miR-29b* and *miR-98* were suggested to play predominant roles in MI. Bioinformatic analysis suggested that *miR-1* regulates myocyte growth, *miR-29b* regulates fibrosis and *miR-98* regulates inflammation^[44].

***miR-1* and *miR-206* and apoptosis:** The potential roles of muscle-specific *miR-1* and *miR-206* and their expression in a rat model of MI have been analyzed. Both miRNAs were significantly increased, while insulin-like growth factor 1 (IGF-1) protein levels were markedly re-

duced. Caspase-3 activity was increased in cells transfected with either *miR-1* or siRNA against IGF-1. Enhanced apoptosis could be therefore induced in cardiomyocytes with a low level of IGF-1 mediated by the post-transcriptional repression caused by *miR-1/miR-206*^[45].

***miR-1* and arrhythmia:** Propranolol was shown to reduce the incidence of arrhythmias in a rat model of MI. Increased expression of *miR-1* was observed in an ischemic myocardium. Administration of propranolol reversed the upregulation of *miR-1* to near control levels, significantly diminishing the incidence of arrhythmias in the first 12 h after MI. The suggested targets for *miR-1* were the cardiac ion channels Cx43 and Kir2.1^[46].

***miR-21*, apoptosis and atrial fibrillation:** The miRNA expression profiling has been performed 6 h after AMI induction in rats. Thirty-eight miRNAs were dysregulated when infarcted area has been compared to non-infarcted heart tissue and 33 in the border zone of the MI when compared to non-infarcted area. *miR-21* was significantly downregulated in the infarcted area but was upregulated in the border zone (6 and 24 h after MI). *miR-21* had a protective effect on ischemia-induced cell apoptosis by targeting PDCD4 and AP-1, which might play critical roles in the early phase of AMI. Importantly, some miRNAs in the non-infarcted area were also differentially expressed 6 h after AMI, suggesting that in addition to dysregulated miRNAs in infarcted tissue and border zone, some miRNAs dysregulated in the remote myocardium might also contribute in the pathophysiological response to AMI^[47]. Another potential role of *miR-21* in the atrial fibrillation (AF) resulted from experimental HF after MI. *miR-21* was upregulated in atrial tissues following MI, along with the dysregulation of target genes sprouty-1, collagen- I, and collagen-III. Anti-*miR-21* treatment reduced atrial *miR-21* expression, decreased AF duration, and reduced atrial fibrous tissue^[48].

***miR-34a* and apoptosis:** In an experimental rat model of MI, the expression of *miR-34a* was highly increased while the expression of aldehyde dehydrogenase 2 (ALDH2) was decreased. Overexpression of *miR-34a* in neonatal rat cardiomyocytes significantly enhanced apoptosis and downregulated ALDH2, suggesting that ALDH2 is a direct target of *miR-34a*. Serum *miR-34a* levels in AMI patients and rats were significantly higher than those in controls^[49].

***miR-101*, *miR-711*, *miR-29b* and fibrosis:** Four weeks after MI induction in rats, examination of miRNAs expression in the peri-infarct area revealed down-regulation of *miR-101a/b*. In rat neonatal CFs, enforced expression of *miR-101a/b* lead to suppression of collagen production and proliferation. These effects were abrogated by co-transfection with antisense inhibitors of *miR-101a/b*. The fibroblast proto-oncogene c-Fos was suggested as a target of *miR-101a*. Anti-fibrotic action of *miR-101a* was

mimicked by silencing c-Fos using siRNA, whereas effect of *miR-101a* in cultured CFs was cancelled by enforced expression of the c-Fos. In rats with chronic MI, four weeks after overexpression of *miR-101a* using adenovirus, remarkable improvement in cardiac performance was observed as well as reduction in interstitial fibrosis and inhibition of c-Fos and TGF- β 1 expression^[50]. Pioglitazone was further shown to increase *miR-711* expression and significantly reduce collagen- I levels similar to CFs, and overexpression of *miR-711* suppressed collagen- I levels. Therefore, pioglitazone may upregulate *miR-711* to reduce collagen- I levels in rats with MI. The *miR-711*-transcription factor SP1-collagen- I pathway may be involved in the anti-fibrotic effects of pioglitazone^[51]. Another fibrosis study has been performed showing that carvedilol protected against myocardial injury induced by AMI. In male rats, cardiac remodeling and impaired heart function were observed 4 wk after MI; the upregulation of COL1A1, COL3A1, and α -smooth muscle actin (α -SMA) mRNA was observed as well as the downregulation of *miR-29b*. COL1A1, COL3A1, and α -SMA were down-regulated and *miR-29b* was upregulated by carvedilol in a dose-dependent manner in rat CFs. Enforced expression of *miR-29b* significantly suppressed COL1A1, COL3A1, and α -SMA expression^[52]. An alternative strategy has also been hypothesized that overexpression of *miR-29b*, which would inhibit mRNAs that encode CF proteins involved in fibrosis, would similarly facilitate progenitor cell migration into the infarcted rat myocardium. The number of GFP-positive cells, capillary density, and heart function were significantly increased in hearts overexpressing *miR-29b*, and downregulation of *miR-29b* with anti-*miR-29b* induced interstitial fibrosis and cardiac remodeling^[53].

Human MI

Microarray analysis: Our group performed genome-wide miRNA expression profiling of human MI (7 d post-MI and 4 wk post-MI) comparing fetal hearts to healthy adult hearts. A number of novel miRNAs were identified as well as some similar expression patterns between human MI and fetal hearts, suggesting involvement of cardiac gene reprogramming also in response after MI. Seven miRNAs were confirmed as dysregulated, including *miR-1*, *miR-133a/b*, *miR-150*, *miR-186*, *miR-210* and *miR-451*^[54].

***miR-29*:** Several miRNAs were shown to be dysregulated in the murine MI model, including *miR-29*. Similarly dysregulation has been observed in human MI, after obtaining border zone of the infarcted cardiac tissue from the patients that received a cardiac transplant^[34].

***miR-1*, *miR-133a/b*, *miR-208a*:** Our group further showed that *miR-1*, *miR-133a/b* and *miR-208* were differentially expressed in human MI and fetal hearts when compared to healthy adults. Time-course changes were observed in human MI, with *miR-208* upregulated across all time points and *miR-1* and *miR-133a/b* downregulated

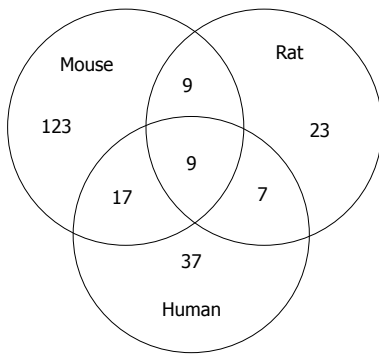


Figure 4 Venn's diagram of number of differentially expressed miRNAs in human myocardial infarction compared to mouse and rat model of myocardial infarction. Experimental data were obtained from microarray analysis performed on mouse model of MI^[32,33], rat model of MI^[43,44] and human MI^[54,58]. MI: Myocardial infarction.

2-7 d after MI. All four miRNAs were downregulated in fetal hearts in comparison to healthy adults. We have also observed some similar patterns of miRNA expression between fetal hearts and MI^[55]. The remote myocardium was also analyzed and compared to healthy adult hearts and the infarcted area. Whereas *miR-1* expression was similar in MI and healthy adults, it was upregulated in the remote myocardium. Downregulation of both *miR-133a* and *miR-133b* was observed in the infarcted tissue as well as in the remote myocardium of patients with MI when compared to healthy adult hearts^[56].

miRNAs and ventricular rupture: Evidence suggests that an intense inflammatory reaction after a MI might contribute to the development of ventricular rupture (VR). In 50 patients with MI (with or without VR), we showed an altered expression of *miR-146a*, *miR-150* and *miR-155* compared to healthy adult hearts. *miR-146a* showed upregulation and *miR-150* and *miR-155* showed downregulation in patients with VR compared to those without. These miRNAs are involved in the regulation of innate immunity and the inflammatory response, providing further evidence that innate immunity resulting in an intense inflammatory reaction plays an important role in the pathogenesis of VR after a MI in humans^[57].

miRNAs and SERCA2: In another study our group also showed 43 dysregulated miRNAs and decreased expression of the protein SERCA2 when infarcted tissue was compared to the corresponding remote myocardium. The prediction of miRNA binding to SERCA2 identified 213 putative miRNAs. miRNA annotation of dysregulated miRNAs revealed 18 functional and 21 disease states that are linked to the cardiovascular diseases. Half of the dysregulated miRNAs were associated with SERCA2. Free-energy binding and flanking regions were defined for 10 upregulated miRNAs (*miR-122*, *miR-320a/b/c/d*, *miR-574-3p/-5p*, *miR-199a*, *miR-140* and *miR-483*). The dysregulation of 9 miRNAs was confirmed (*miR-21*, *miR-122*, *miR-126*, *miR-1*, *miR-133*, *miR-125a/b* and *miR-98*)^[58].

Comparison of the number of differentially ex-

pressed miRNAs from microarray studies performed on human MI^[54,58], on mouse^[32,33] and rat model of MI^[43,44] is summarized in Figure 4. Only a small proportion of differentially expressed miRNAs overlaps between three different species, these are *let-7b*, *let-7f*, *miR-26b*, *miR-126-3p*, *miR-126-5p*, *miR-195*, *miR-199a-3p*, *miR-214* and *miR-451*. All these microarray analyses were performed at different time point post-MI. However, comparison has been performed including any dysregulated miRNA at any time point post-MI within species.

Circulating miRNAs

Serum and exosome miRNAs as AMI biomarkers:

In patients with AMI as well as in patients with angina pectoris (AP), significant increase in serum levels was observed for *miR-1* and *miR-133a*. *miR-133a* has been recognized as a circulating marker for cardiomyocyte death, because its elevated expression is observed in patients with an injured myocardium. Using an experimental mouse model, it was further identified that significant reduction in levels of *miR-1*, *miR-133a*, *miR-208a* and *miR-499* occur in the infarcted myocardium. After stimulation of cardiomyoblasts, exosome fraction of the culture medium was obtained. The measurement of *miR-133a* was performed. Significant elevation of *miR-133a* was observed upon the detection of cell death^[59]. Using an *in vitro* cardiac cell necrosis model, it was shown that cardiac *miR-1* was released into the culture media 20 min after induction, where it is stable for at least 24 h. The amount of *miR-1* released was related to the number of necrotic cardiac myocytes. Furthermore, a time-course study of serum *miR-1* in a rat model included time points at 1, 3, 6, 12, 24 h, and 3, 7, 14, 21 and 28 d after AMI. Serum *miR-1* levels were increased after AMI with a peak at 6 h, returning to the basal level 3 d after AMI, and showed a strong positive correlation with MI size. Research in humans has shown that in 31 patients with AMI, *miR-1* was significantly increased within 24 h after AMI and showed a positive correlation with serum creatine kinase-MB, suggesting its relationship to MI size also occurs in humans. At days 3 and 7, the serum levels returned to baseline^[60]. In another study, serum samples were taken from 117 patients with AMI, 182 patients with AP and 100 age- and gender-matched controls. Six serum miRNAs, *miR-1*, *miR-134*, *miR-186*, *miR-208*, *miR-223* and *miR-499*, were identified as AMI biomarkers and presented significant differences between the AMI and AP cases. *miR-208* and *miR-499* showed higher expression in the AP cases than in the AMI cases^[61].

Serum and exosome miRNAs and prognosis after MI:

Using sera collected a median of 18 d after AMI onset, miRNAs were screened in 21 patients who experienced development of HF within 1 year after AMI and in 65 matched controls. *miR-192*, *miR-194* and *miR-34a*, all p53-responsive miRNAs, were coordinately increased, particularly in exosomes. The serum level of *miR-192* was significantly upregulated in AMI patients with develop-

ment of ischemic HF. *miR-194* and *miR-34a* expression levels were significantly correlated with the left ventricular (LV) end-diastolic dimension 1 year after AMI^[62]. The prognostic impact of circulating miRNAs in patients who survived AMI was also analyzed by a high-throughput array consisting of 667 miRNAs. Eleven miRNAs were differentially expressed in the serum from patients at high-risk for cardiac death, and a subset of circulating miRNAs might be predictive for cardiac death in post-AMI patients. Serum levels of *miR-155* and *miR-380** were higher in patients who experienced cardiac death within 1 year after discharge^[63].

Whole blood miRNAs as AMI biomarkers: After performing miRNA expression profiling in peripheral whole-blood samples of patients with AMI, 121 dysregulated miRNAs have been identified. These miRNAs possess a unique signature of 20 miRNAs predicting AMI with 96% specificity, 90% sensitivity and 93% accuracy. *miR-30c* and *miR-145* levels were expressed in correlation with infarct size, which was estimated by release of Troponin T (TnT). Identification of miRNAs that is not based solely on the release of miRNAs from a necrotic myocardium is important for understanding active processes involved in the pathogenesis of MI (inflammation, plaque, rupture and vascular injury). Dysregulated miRNAs in AMI might be equally derived from other cellular populations that play an active role in AMI pathophysiology^[64]. To characterize temporal expression patterns of miRNAs in MI, another study was performed with miRNA expression levels measured at multiple time points (0, 2, 4, 12, 24 h after the initial presentation) in patients with acute MI. A subset of miRNAs was found to be significantly dysregulated both at the initial presentation and during the course of AMI. Novel miRNAs that are dysregulated early during MI were identified (*miR-1915* and *miR-181c**)^[65].

Whole blood and plasma miRNAs as AMI biomarkers: The whole blood and plasma samples were obtained from 51 AMI patients and compared with 28 control subjects. Sample collection from AMI patients was performed within 24 h and 7 d after the onset of AMI. In plasma as well as in whole blood from AMI patients, elevated *miR-133* and *miR-328* levels was observed. Seven days after onset of AMI symptoms increased circulating *miR-133* and *miR-328* levels returned to control levels. There has also been observed a correlation between cardiac Troponin I (cTnI) and circulating *miR-133* or *miR-328*^[66].

Plasma miRNAs as AMI biomarkers: In AMI rats, plasma samples were taken at 1, 3, 6, 12 and 24 h. At these time points, measurement of levels of *miR-1*, *miR-133a*, *miR-499* and *miR-208a* has been performed. All these miRNAs were significantly increased, at least at one time point. *miR-208a* was undetectable at time 0 h, increased 1 h after AMI and reached its peak at 3 h. At

time point 6-12 h, it began decreasing and at 24 h it was undetectable. At time point 1-3 h, *miR-1*, *miR-133a* and *miR-499* were elevated, at 3-12 h reached their peak and at 12-24 h finally decreased. *miR-1*, *miR-133a*, *miR-499* and *miR-208a* were present at very low levels or were absent in the plasma of healthy people, but were substantially higher in the plasma of 33 AMI patients compared with that of patients with other cardiovascular diseases, whereas *miR-208a* remained undetectable in patients with non-AMI heart diseases^[67]. In another study, miRNAs were analyzed in human plasma, mouse plasma and mouse cardiac muscle. A microarray analysis showed 20 upregulated and 14 downregulated miRNAs in 17 healthy donors compared with 33 patients with AMI. *miR-1*, *miR-133a/b* and *miR-499-5p* were upregulated and *miR-122* and *miR-375* were downregulated 6-12 h after MI onset. Five days later, all miRNAs were back to basal plasma levels, except that *miR-122* was lower than in controls through day 30. Compared to cTnI, peak expression was observed at a similar time in MI patients for *miR-1* and *miR-133a/b*, but *miR-499-5p* showed a slower time course. In mice, the pattern of upregulated miRNAs was similar to that in MI patients, but reciprocal expression was observed in cardiac tissue 3-6 h after MI^[68]. *miR-1* level was measured in a larger cohort of patients (159) with or without AMI. In the plasma from AMI patients, *miR-1* was significantly increased when compared with non-AMI patients. Its levels decreased to normal after medication. Statistical analysis revealed that elevated levels of circulating *miR-1* were not in correlation to patient characteristics (established biomarkers for AMI, concurrent disease as are blood pressure and diabetes mellitus or either age or gender)^[69]. Increased *miR-1* and decreased *miR-126* expression were consistently observed in the plasma from 17 patients with AMI compared with 25 healthy subjects. cTnI, *miR-1* and *miR-126* expression levels showed the same trend^[70]. *miR-1*, *miR-133a*, *miR-208b* and *miR-499* were further compared to cTnT for diagnostic value. Study has been performed on 67 patients with AMI and 32 healthy volunteers. The levels of all plasma miRNAs were significantly higher in AMI patients than in healthy volunteers. At the time of hospital discharge of AMI patients, expression of the cardiac-specific miRNAs was reduced to near baseline levels. However, it has turned out that for the diagnosis of AMI, the four plasma miRNAs were not superior to cTnT^[71]. In another study, plasma samples were obtained from 18 patients with AMI and 30 healthy adults. In this cohort of samples, *miR-30a*, *miR-195* and *let-7b* levels were examined. At time points 4 h, 8 h and 12 h after the onset of AMI, circulating *miR-30a* was highly elevated. In AMI patients, *miR-195* was also highly expressed, when compared to control, but only at time points 8 h and 12 h. Through all the time points, *let-7b* was lower in AMI patients when compared to control samples. All three investigated circulating miRNAs, *miR-30a*, *miR-195* and *let-7b*, showed the peak expression at 8 h and were of significant diagnostic value for AMI^[72].

Plasma miRNAs for differentiating MI: Plasma concentrations of cardiac-enriched *miR-208b* and *miR-499* were measured in a case-control study of 510 MI patients and 87 healthy controls. *miR-208b* and *miR-499* showed elevated expression in patients with MI and were nearly undetectable in healthy controls. In 397 patients with ST-elevation MI (STEMI), miRNAs had higher concentrations than in 113 patients with non-STEMI (NSTEMI)^[73].

Plasma miRNAs for differentiating MI from other cardiovascular diseases: In all individuals with AMI, the concentration of plasma *miR-499* was shown to be increased; however it was below the detection limit in other groups of patient [control, chronic HF (CHF), and unstable AP]^[74]. The expression level of plasma *miR-133a* has been analyzed in 13 AMI patients, 176 AP patients and 127 control subjects for its relationship to the severity of coronary stenosis. The results showed that circulating *miR-133a* levels were significantly increased in AMI patients in a time-dependent manner, achieving a peak at 21.6 ± 4.5 h after the onset of AMI symptoms and showed a similar trend as the level of plasma cTnI. Importantly, the levels of circulating *miR-133a* positively correlated with the severity of coronary artery stenosis^[75]. Another study showed that plasma levels of *miR-1*, *miR-133a*, *miR-208b* and *miR-499* (muscle- or cardiac-specific or enriched miRNAs), *miR-21* and *miR-29b* (fibrosis-related miRNAs) *miR-146*, *miR-155*, *miR-223* (leukocyte-associated miRNAs) are associated with different degrees of cardiac injury as are AMI, acute HF, diastolic dysfunction and even viral myocarditis. In the plasma of 32 patients with AMI, *miR-208b* and *miR-499* were highly elevated compared with control subjects and both correlated with plasma cTnT levels. Both miRNAs also showed significant but milder elevation in viral myocarditis. However, in patients with acute HF, only *miR-499* showed significant elevation, whereas no significant change was observed in diastolic dysfunction^[76]. Another study group consisted of 17 patients with AMI, 4 with stable coronary artery disease (CAD) and 5 with no history of CAD. Expression of *miR-423-5p*, *miR-208* and *miR-1* was measured in plasma before percutaneous coronary intervention (PCI), at 6, 12 and 24 h. In stable CAD, the expression of *miR-1*, *miR-208a* and *miR-423-5p* did not show any significant differences at any time point. There was a higher number of *miR-423-5p* copies in patients with AMI before the PCI. However, 6, 12 and 24 h after PCI, the expression levels were similar to the control group and significantly lower than the baseline level. The expression levels of *miR-1* and *miR-208a* were not significantly different from the control group^[77]. In another study, the increased expression levels of *miR-1*, *miR-21*, *miR-133a*, *miR-423-5p* and *miR-499-5p* has been showed in plasma of 92 patients with NSTEMI compared to 99 age-matched healthy control subjects. *miR-499-5p* and *miR-21* showed increased expression in NSTEMI compared to 81 patients with CHF. *miR-499-5p* also showed good diagnostic accuracy in differentiating patients with NSTEMI and CHF^[78]. Takotsubo cardio-

myopathy (TTC) is clinically indistinguishable from AMI, and no established biomarkers are available for the early diagnosis of TTC and differentiation from AMI. After miRNA profiling, eight miRNAs were selected for verification in 36 patients with TTC, 27 patients with AMI and 28 healthy controls. Upregulation of *miR-16* and *miR-26a* was confirmed in patients with TTC compared with healthy subjects, and upregulation of *miR-16*, *miR-26a* and *let-7f* was observed in TTC compared with MI patients. Compared with healthy controls, *miR-1* and *miR-133a* showed upregulation in patients with MI, and *miR-133a* was substantially increased in patients with MI when compared with TTC. A unique signature comprising *miR-1*, *miR-16*, *miR-26a* and *miR-133a* differentiated TTC from healthy subjects and from MI patients^[79].

Plasma miRNAs and prognosis after MI: Plasma *miR-1*, *miR-21*, *miR-29a*, *miR-133a* and *miR-208* were measured in 12 age-matched reference controls and 12 post-MI patients from day 2 through day 90 post-MI. After MI, a progressive increase of LV end-diastolic volume was accompanied by time-dependent changes in specific miRNAs. Two days post-MI, *miR-21* decreased and 5 d post-MI increased. At later time points its expression level reached the control values. Similarly, at day 5 post-MI, *miR-29a* increased and then decreased to the control level at later time points. *miR-208* showed elevated expression at day 5 post-MI and did not show any decrease up to day 90 post-MI^[80]. *miR-1*, *miR-208b* and *miR-499-5p* were further measured in plasma samples from 424 patients for discrimination of a clinical diagnosis of MI and for association with 30-d mortality and for diagnosis of HF. Discrimination of MI was accurate for *miR-208b* and *miR-499-5p* but was considerably lower than for TnT. Increased miRNA levels were strongly associated with an increased risk of mortality or heart failure within 30 d for *miR-208b* and *miR-499-5p*, but the association was lost when adjusting for TnT^[81]. In another study, circulating miRNAs were measured in 90 patients after AMI and several miRNAs were identified as potentially involved in LV remodeling. *miR-150* was downregulated in patients with remodeling compared with patients without. *miR-150* outperformed B-type natriuretic peptide (BNP) to predict remodeling and reclassified 54% of patients misclassified by BNP and 59% of patients misclassified by a multi-parameter clinical model^[82]. Furthermore, plasma samples from 150 patients with AMI were obtained for determination of the levels of *miR-16*, *miR-27a*, *miR-101* and *miR-150*. A combination of the four miRNAs improved the prediction of LV contractility based on clinical variables. Patients with low levels of *miR-150* or *miR-101* and elevated levels of *miR-16* were at high risk for impaired LV contractility. The four-miRNA panel reclassified a significant proportion of patients, with a net reclassification improvement of 66%^[83].

Plasma miRNAs and prospective study for MI: The association between baseline levels of miRNAs, the in-

cidence of MI, and the cellular origin of miRNAs was analyzed in 820 participants with 19 candidate miRNAs. Three miRNAs were consistently and significantly related to the incidence of MI; *miR-126* showed a positive association, *miR-223* and *miR-197* were negatively related to the risk of disease. Control group consisted of healthy volunteers, in who limb I/R was performed by thigh cuff inflation. After obtaining plasma samples at baseline, 10 min, 1 h, 5 h, 2 d, and 7 d, miRNA expression was analyzed. Six distinct miRNA clusters were identified by computational analysis, and one of them consisted of all miRNAs that were related to the risk of a future MI. This cluster included miRNAs predominantly expressed in platelets and its characteristic was activation 1 h post-I/R (early) and activation 7 d post-I/R (sustained). Platelets were suggested as being a major contributor to this miRNA expression pattern, since in subjects with a subsequent MI, dysregulated patterns of circulating miRNAs occurred with endothelium-enriched *miR-126*^[84].

Plasma and urine miRNAs: In a pig I/R model, *miR-1*, *miR-133a* and *miR-208b* increased rapidly in plasma with a peak at 120 min, while *miR-499-5p* remained elevated longer. In humans, 25 patients with MI revealed that all four miRNAs were increased in plasma, with a peak at 12 h. Peak values of *miR-208b* correlated with peak troponin levels. *miR-1* and *miR-133a* both correlated strongly with renal elimination, which was confirmed by detection of *miR-1* and *miR-133a*, but not *miR-208b* or *miR-499-5p*, in the urine^[85].

Urine miRNAs: Blood protein MI biomarkers (creatinine phosphokinase-muscle band, TnT and TnI) are not typically filtered into urine. Urine *miR-1* was quickly increased in rats with a peak at 24 h after AMI and returned to the basal level 7 d after AMI. No *miR-208* was observed in normal urine; however, *miR-208* was easily detected in urine from rats with AMI. Serum exosomes from rats after AMI were isolated and injected into the circulating blood of normal rats; urine *miR-1* was significantly increased in the exosome-injected animals. The levels of urine *miR-1* were also significantly increased in patients with AMI^[86].

In summary, it has been shown that miRNAs may be useful circulating biomarkers for the diagnosis of AMI, differentiating them from other cardiovascular diseases and prognoses after MI. However, two studies have shown that miRNAs are not useful circulating biomarkers for some aspects of MI, (1) for prognosis of patients with STEMI; or (2) for an incidence of LV remodeling 1 year after anterior AMI^[87,88].

Therapeutic opportunities

All miRNAs as potential therapeutic targets were tested in mouse or rat models of MI. miRNAs and different therapeutic approaches analyzed in mouse model of MI are summarized in Figure 5 and miRNAs and different therapeutic approaches analyzed in rat model of MI are

overviewed Figure 6.

***miR-181a* and skeletal myoblast transplantation in rats with MI:** A lentiviral siRNA against the loop region of *miR-181a* was shown to upregulate the skeletal myoblast (SKM) differentiation repressor Hox-A11 and reduce arrhythmias following SKM transplantation into ischemic myocardium of rats. Engraftments of SKMs with *miR-181a* knockdown improved cardiac function and significantly decreased the arrhythmogenic effect of SKM transplantation in rats with experimental MI^[89].

***miR-210* and treatment of ischemic heart disease:** *miR-210* was highly expressed in mouse cardiomyocytes that survived 48 h after hypoxia exposure compared with apoptotic cardiomyocytes. Mice receiving a *miR-210* precursor showed significant improvement of LV fractional shortening after 8 wk. Histological analysis showed decreased cellular apoptosis and increased neovascularization. Two target genes involved in inhibition of angiogenesis/vascular remodeling and induction of apoptosis, Ephrin-A3 and Ptp1 (non-receptor phospho-tyrosine protein phosphatase), were confirmed. It has been shown that *miR-210* can improve angiogenesis, inhibit apoptosis and improve cardiac function in a mouse model of MI^[90].

Phosphoinositide-3-kinase-regulated miRNA and mRNA: Activation of phosphoinositide-3-kinase (PIK3) is considered a new strategy for the treatment of heart failure and MI. To identify cardiac-selective miRNAs and mRNAs that mediate the protective properties of PIK3, experimental mice were used and identified growth factor receptor-bound protein (Grb14) gene expression that positively correlated with cardiac function. Grb14 is highly expressed in the mouse heart compared with other tissues. Three miRNAs were also highly correlated with Grb14, namely *miR-210*, *miR-34a* and *miR-222*^[91].

Tanshinone and miR-1: Accumulating evidence suggests that tanshinone II A can reduce the ischemic area and improve cardiac function and has been shown to suppress *miR-1* expression. Using a rat model of MI, tanshinone II A was administered daily for 7 d before MI and lasted for 3 mo following MI. Tanshinone II A was shown to relieve ischemia-induced injury, decrease the elevated *miR-1* levels in ischemic and hypoxic cardiomyocytes, and consequently restored the normal level of the *miR-1* target Cx43. In ischemic and hypoxic cardiomyocytes, tanshinone II A also inhibited activated p38 MAPK, SRF and MEF2^[92].

Ivabradine and *miR-1* and *miR-133a*: Ivabradine is a selective inhibitor of the hyperpolarization-activated, cyclic nucleotide-gated pacemaker current. Its effect on electrophysiological remodeling of myocytes from post-MI rats was observed as a decrease in the transcription of HCN4, a target of *miR-1* and *miR-133a*. Both, *miR-1* and *miR-133* were significantly elevated in myocytes. The

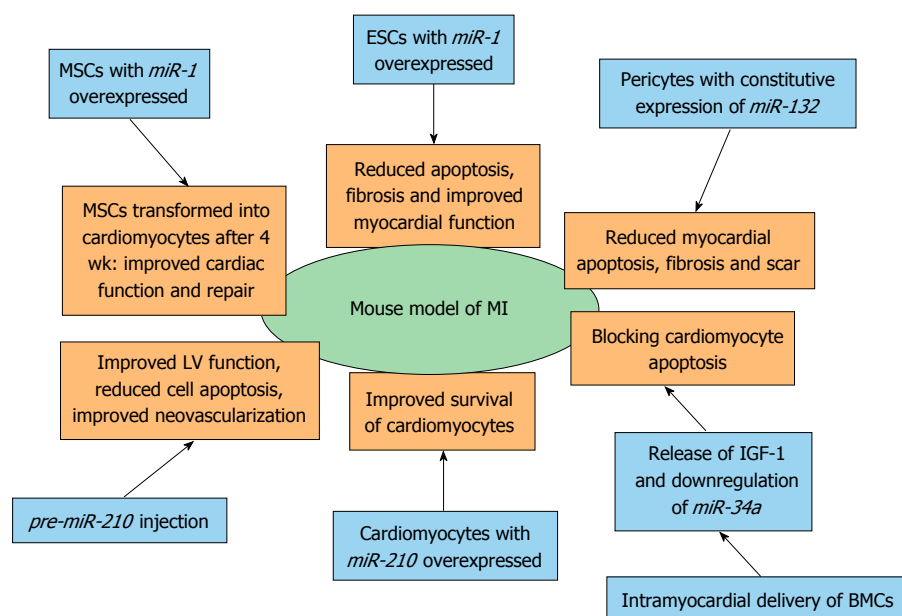


Figure 5 Therapeutic opportunities of miRNAs in myocardial infarction identified by using mouse model of myocardial infarction. BMC: Bone marrow cell; ESC: Embryonic stem cell; LV: Left ventricle; MSC: Mesenchymal stem cell; MI: Myocardial infarction; IGF-1: Insulin-like growth factor 1.

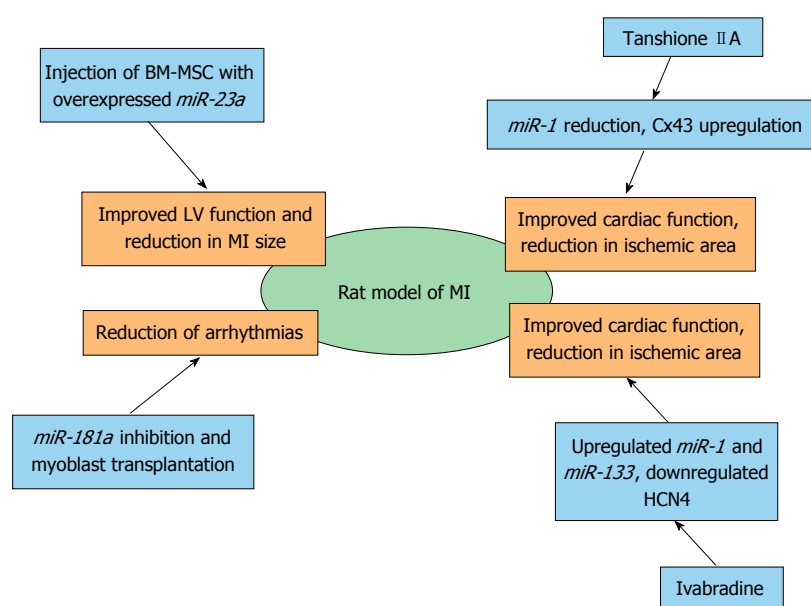


Figure 6 Therapeutic opportunities of miRNAs in myocardial infarction identified by using rat model of myocardial infarction. BM-MSC: Bone marrow-mesenchymal stem cell; LV: Left ventricle; MI: Myocardial infarction; Cx43: Gap junction alpha-1 protein; HCN4: Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4.

beneficial effects of ivabradine may be due to the reversal of electrophysiological cardiac remodeling by reducing the overexpressed HCN channels in post-MI rats^[93].

Embryonic stem cells and miRNAs: Embryonic stem cells (ESC) with overexpressed *miR-1* were transplanted into the infarcted myocardium of experimental animals, and reduced apoptosis was subsequently observed 4 wk post-MI. A significant elevation in p-Akt levels and diminished PTEN levels were also observed. The mice also had a significant improvement in some physiological car-

diac functions^[94]. The same author investigated whether overexpression of *miR-1* in ESCs would enhance cardiac myocyte differentiation following transplantation into the infarcted myocardium. Two weeks after transplantation into the border zone of the infarcted heart, cardiac myocyte differentiation, adverse ventricular remodeling, and cardiac function were assessed. Overexpression of *miR-1* in transplanted ESCs protected the host myocardium from MI-induced apoptosis. A significant reduction in interstitial and vascular fibrosis was observed as well as significantly improved heart function^[95].

Mesenchymal-stem cells and miRNAs: One week after MI, mice were intramyocardially injected at the heart infarcted zone with *miR-1*-transduced mesenchymal-stem cells (MSCs). At 4 wk post-transplantation, transplanted MSCs were able to differentiate into cardiomyocytes in the infarcted zone. Cardiac function with the *miR-1*-transduced MSCs was significantly improved, and treatment of MSCs expressing *miR-1* was more effective for cardiac repair, most likely by enhancing cell survival and cardiac myocyte differentiation compared with the MSCs without *miR-1*^[96]. *In vitro* co-culture between cardiomyocytes and MSCs has been established to test whether MSCs deliver *miR-210* to host cardiomyocytes; this showed co-localization of *miR-210* with the gap-junction protein Cx43. *miR-210* has been proposed to be transferred through gap junctions. Higher survival rates of cardiomyocytes co-cultured with MSCs was observed with concomitant expression of caspase-8 associated protein 2 (CASP8AP2) suggesting that *miR-210* translocates from MSCs to protect host cardiomyocytes. Direct transfer of pro-survival *miR-210* from MSCs to host cardiomyocytes led to a functional recovery of the ischemic hearts of the experimental animals^[97]. The clinical application of MSC-based therapy is restricted because of the poor survival of implanted cells. Using a tumor necrosis factor α -(TNF- α)-induced bone marrow (BM)-MSC injury model and a rat MI, it has been shown that *miR-23a* was involved in TNF- α -induced BM-MSC apoptosis through regulating caspase-7 and that the injection of BM-MSCs overexpressing *miR-23a* could improve LV function and reduce the infarct size in the rat MI model^[98].

Bone marrow cells and *miR-34a*: Cell therapy with bone marrow cells (BMC) can improve the recovery of cardiac function after ischemia. Intra-myocardial delivery of BMCs in infarcted mice has been shown to regulate the expression of miRNAs in the heart and downregulate the expression of *miR-34a*, a pro-apoptotic miRNA. Transplanted BMCs regulate cardiac miRNAs by paracrine mode and thus contribute to the protective effects. IGF-1 inhibits the *miR-34a* processing and is released by BMCs, thereby blocking apoptosis in cardiomyocytes^[99].

Pericytes and *miR-132*: Pericytes are key regulators of vascular maturation and therapeutic activity, and mechanistic targets of saphenous vein-derived pericyte progenitor cells (SVPs) have been investigated using a mouse MI model. Transplantation of SVPs into the peri-infarct zone of mice attenuated LV dilatation, reduced myocardial scar, cardiomyocyte apoptosis and interstitial fibrosis, and blood flow and neovascularization. *miR-132* was constitutively expressed and secreted by SVPs and markedly upregulated. Ras-GTPase activating protein and methyl-CpG-binding protein 2 were shown to be targets of *miR-132*. *miR-132* inhibition decreased SVP capacity to improve contractility, reparative angiogenesis, and interstitial fibrosis in infarcted hearts^[100].

Telocytes and miRNAs: Telocytes (TCs) are a novel

type of interstitial cell recently discovered in the myocardium. Rat experimental MI was investigated using electron microscopy, immunocytochemistry and analysis of several pro-angiogenic miRNAs that provided evidence for TC involvement in neo-angiogenesis after MI. TCs contain measurable quantities of angiogenic miRNAs (*let-7e*, *miR-10a*, *miR-21*, *miR-27b*, *miR-100*, *miR-126-3p*, *miR-130a*, *miR-143*, *miR-155* and *miR-503*)^[101].

Bioinformatics analysis

Rationally designed bioinformatic analysis combined with experimental approaches to screen key therapeutic members of the IUPHAR database was conducted, following establishment of the whole genome protein interaction network and a comprehensive topological assessment. The number of validated and confidently predicted miRNAs regulating each gene encoding an ion channel or a gap junction protein was counted. Cx43 showed more intensive miRNA regulation compared with other ion channel and gap junction proteins^[102].

My-Inflamome: One of crucial processes in cardiac repair after MI is inflammation. In a study, a network has been established that enhances understanding of the inflammatory responses and its interaction network in human MI. The network is called My-Inflamome and it assembles protein interactions that are associated to inflammation and related to prognosis after MI. Classification models were established based on microarray data of blood samples from patients after MI with various disease consequences. Significant associations were experimentally verified. Different biological processes included in the heart repair are organized into modules. Small set of miRNAs is also included in modules that are significantly associated with transcriptional regulation^[103].

My-DTome: Another computational approach has been performed. It is based on different drug and protein interaction and it is called My-DTome (it is assembling the MI drug-target). It is also consisted of modules, which are related to the important molecular processes and pathways and to potential therapeutic approaches in MI that might be miRNAs-regulated. Non-cardiovascular drugs may also possess the cardiovascular effects and this systemic insight was established. This network might represent the basis for an investigation of new multidrug treatment and new targets MI^[104].

Polymorphisms in miRNA binding sites

After searching across dbSNP and TargetScan, 10 SNPs in potential miRNA binding sites of 8 RAAS-related genes were identified and genotyped for risk for MI and blood pressure. It was found that nine SNPs in seven genes were prevalent. Of the nine SNPs, four in three genes were associated with blood pressure. The rare allele of the mineralocorticoid receptor (NR3C2) (SNP rs5534) was associated with a twofold increased risk of MI in men younger than 50 years of age. The reduction in miR-induced repression of gene expression was demonstrated^[105].

CONCLUSION

In recent years miRNAs have been recognized as promising therapeutic, diagnostic and prognostic factors in the field of cardiovascular diseases. The usefulness of circulating miRNAs in the diagnosis and prognosis of MI has been established through numerous studies. Moreover, the therapeutic potential of miRNA has been established, especially in field of stem cell research. Heart tissue expression patterns examined in numerous experimental animals still need to be confirmed on human MI. Much more work is necessary before establishing routine use of miRNAs in clinical diagnosis, prognosis and therapy; however, the current findings are encouraging.

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Ventricular tachycardia mapping and ablation in arrhythmogenic right ventricular cardiomyopathy/dysplasia: Lessons Learned

Cory M Tschabrunn, Francis E Marchlinski

Cory M Tschabrunn, Harvard-Thorndike Electrophysiology Institute, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, United States

Francis E Marchlinski, Cardiac Electrophysiology Program, Hospital of the University of Pennsylvania, Philadelphia, PA 19102, United States

Author contributions: Tschabrunn CM wrote the manuscript; Marchlinski FE reviewed and edited the manuscript.

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Correspondence to: Francis E Marchlinski, MD, Cardiac Electrophysiology Program, Hospital of the University of Pennsylvania, Founders 9 Pavilion, Philadelphia, PA 19102, United States. francis.marchlinski@uphs.upenn.edu

Telephone: +1-215-6626005 Fax: +1-215-6622879

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has allowed clinician scientists to better characterize the arrhythmia mechanism and develop the necessary strategies to perform successful catheter ablation. Early in this experience, catheter ablation was considered a limited and largely unsuccessful treatment for patients experiencing painful and recurrent defibrillator therapy. Through our increased understanding of the disease process, catheter ablation has evolved to become an effective and preferred therapy for a majority of these patients. Our understanding of the disease and necessary approaches to provide successful treatment continues to evolve as the clinical experience grows. This article will review these important insights from the electrophysiology laboratory and how application of this knowledge has facilitated the development of a methodical approach to successfully perform ventricular tachycardia ablation in patients with ARVC/D.

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Abstract

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is primarily believed to be an inherited cardiomyopathy that subsequently results in significant myocardial fibrosis. The arrhythmogenic consequences that result from the development of fibrosis are similar to other nonischemic cardiomyopathies, but the unique endocardial-epicardial disease process of ARVC/D requires a specialized approach for arrhythmia treatment in the electrophysiology laboratory. Although the association between ARVC/D and development of ventricular arrhythmias has become increasingly clear over the last 2 decades, our understanding of the arrhythmia mechanisms, underlying electrophysiologic substrate, and treatment strategies were significantly limited. Prospective studies performed in the electrophysiology laboratory allowed detailed characterization of the electrophysiologic and electroanatomic substrate underlying ventricular tachycardia in patients with ARVC/D. This

Key words: Arrhythmogenic right ventricular cardiomyopathy/dysplasia; Ventricular tachycardia; Mapping; Ablation

Core tip: This review article evaluates seminal insights derived from the electrophysiology laboratory and the lessons learned to develop a methodical approach that can utilized to successfully perform ventricular tachycardia ablation in patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia.

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INTRODUCTION

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a fascinating disease that continues to challenge clinicians and scientists since it was first described in 1728^[1]. Though several hypotheses have been proposed for the underlying cause of ARVC/D, it is primarily believed to be an inherited cardiomyopathy resulting from gene mutations that encode desmosomal proteins, the organelle responsible for cell-cell adhesion. Desmosomal dysfunction in patients with ARVC/D leads to inadequate cell adhesion and subsequent myocyte detachment and apoptosis^[2,3]. The accumulation of fibrous and adipose tissue predominantly affects the right ventricular free wall and typically extends inward from the epicardium toward the endocardial surface^[4,5]. Although this underlying process of ventricular scarring is unique to ARVC/D, the arrhythmogenic consequences that result from the development of fibrosis are similar to other nonischemic cardiomyopathies. The extensive right ventricle (RV) fibrosis results in inhomogeneous conduction with slow and discontinuous electrical propagation in sinus rhythm that serves as the substrate for ventricular arrhythmias. Although the association between ARVC/D and the subsequent development of ventricular arrhythmias had become increasingly clear following early clinical cohort reports, the characterization of arrhythmia mechanisms, the underlying electrophysiologic substrate, and treatment strategies were, until recently, poorly understood and limited.

Much of our understanding of the electrophysiologic and electroanatomic substrate underlying ventricular tachycardia (VT) in patients with ARVC/D was derived from studies performed within the electrophysiology laboratory. Through this experience, much has been learned about the arrhythmia mechanisms and strategies required to facilitate successful catheter ablation. The ability to localize and define the associated abnormalities essential for VT enhanced the effectiveness of catheter ablation procedures. What was once considered a treatment of last resort has now become the preferred therapy for most patients with documented ventricular arrhythmias. In addition, assessment of the anatomic substrate during electrophysiology procedures has shed important light on controversies pertaining to disease pathogenesis.

This review article will evaluate these seminal insights derived from the EP lab and the lessons learned to develop a methodical approach that can be utilized to successfully perform VT ablation in patients with ARVC/D.

PATIENT SELECTION FOR CATHETER ABLATION

Patients are typically diagnosed with ARVC/D after clinical manifestation of signs or symptoms during the second to fifth decade of life. We recommend implantable cardioverter-defibrillator (ICD) implantation to a majority of patients due to the high incidence of ventricular

arrhythmias associated with the disease after a definitive diagnosis is made according to the task-force criteria guidelines^[6]. Although ICD therapy is routine, management of recurrent VT with frequent device therapy can be difficult. Antiarrhythmic medications are often poorly tolerated and may only provide incomplete VT control. Inadequate arrhythmia control and the use of multiple antiarrhythmic medications is particularly debilitating for these young, and often physically very active patients.

Although techniques used in catheter ablation of VT in patients with ARVC/D have evolved over the last decade, outcomes are still inconsistent, ranging from 50%-90%^[7]. This is likely the result of a number of different mapping and ablation strategies with variable endpoints, follow-up assessment, and operator experience^[8-13]. In our experience at the University of Pennsylvania, a comprehensive ablation strategy that targets both the endocardial and epicardial substrate with elimination of abnormal electrograms and all inducible VT provides long-term drug-free arrhythmia control in a large majority of patients. For this reason, we offer catheter ablation to all patients with recurrent VT refractory or intolerant to medical therapy.

INSIGHTS FROM ELECTROANATOMIC MAPPING: DEFINING THE ELECTROPHYSIOLOGIC AND ELECTROANATOMIC SUBSTRATE UNDERLYING VT IN ARVC/D

Endocardial substrate

Advances in 3D electroanatomic mapping enabled a more thorough understanding of the complex electrophysiologic substrate in patients with ARVC/D and VT. Abnormal RV endocardial regions can be localized with electroanatomic mapping by identifying regions of low bipolar RV endocardial voltage (< 1.5 mV) and long-duration, low-amplitude, fractionated potentials. These key areas identified have been correlated to relevant histopathologic findings (myocyte loss with fibrofatty replacement) and critical VT circuits confirming the involvement of these areas in the arrhythmogenic mechanism^[14]. The endocardial distribution of electroanatomic scar in patients with VT and ARVC/D typically extends from the tricuspid valve and/or the pulmonary valve to the RV free wall. Low-voltage abnormalities can also be found on the septal aspect of the perivalvular region(s), but typically does not include the RV apex (Figure 1)^[15].

Although ARVC/D is known to primarily involve the RV, involvement of the left ventricle (LV) is more frequent than previously recognized. LV abnormalities have been documented with electroanatomic mapping and typically involve the basal perivalvular region, which is characteristic of other non-infarct related cardiomyopathies (Figure 2)^[15]. Consideration of endocardial LV involvement is of particular importance if right bundle branch block VTs

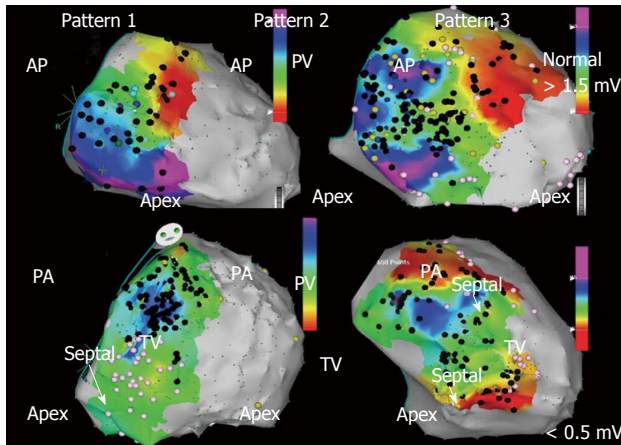


Figure 1 Bipolar right ventricle endocardial voltage maps demonstrating characteristic patterns of low voltage (< 1.5 mV) regions identified in patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia and ventricular tachycardia in anterior and posterior views. Peritricuspid (pattern 1), peripulmonic (pattern 2), or more extensive involvement extending from both valvular regions (pattern 3) is shown. Distribution of abnormal electrograms is predominantly free wall. Right ventricle apex is spared, and septal involvement is frequently identified (arrows). Adapted from Marchlinski *et al.*^[15] with permission. AP: Anterior; PA: Posterior.

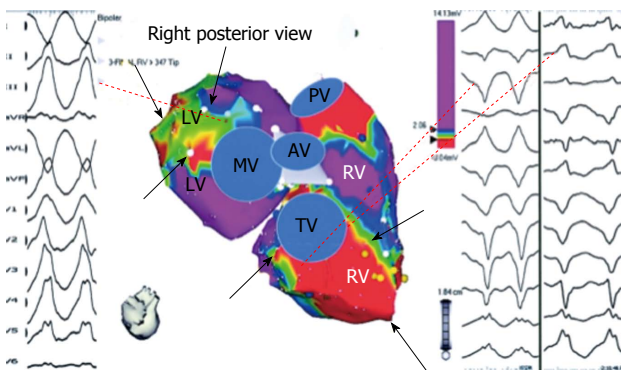


Figure 2 Bipolar right ventricle and left ventricle endocardial voltage maps highlighting location of abnormal endocardium and origin of ventricular tachycardia in a patient with right ventricle cardiomyopathy and ventricular tachycardia. Electroanatomic abnormalities include both the tricuspid and mitral valves from tricuspid and mitral valves (black arrows). Origin of ventricular tachycardia (VT) based on activation and pace mapping was perivalvular mitral for the RBBB VT and perivalvular tricuspid valve for LBBB VT (dashed lines). Adapted from Marchlinski *et al.*^[15] with permission. RV: Right ventricle; LV: Left ventricle.

with positive R waves in the precordial leads are seen as this suggests an LV VT exit site of interest.

Epicardial substrate

Despite periprocedural advances with irrigated ablation catheter technology and criteria to identify RV endocardial bipolar electroanatomic voltage abnormalities, the endocardial ablation approach provides only modest long-term arrhythmia freedom^[15]. The epicardial to endocardial scarring process associated with ARVC/D often results in a more extensive abnormal epicardial substrate that may not be amendable to endocardial ablation alone. Insights from percutaneous epicardial map-

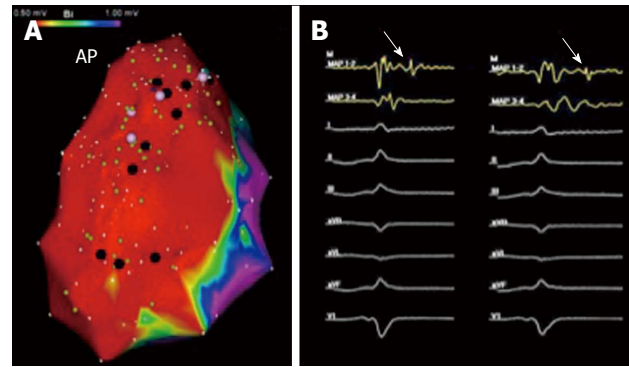


Figure 3 Epicardial right ventricle bipolar voltage map and isolated late potentials in sinus rhythm. A: Demonstrates significant epicardial bipolar voltage abnormalities (< 1.0 mV) over the right ventricle free wall. The black tags on the electroanatomical map represent areas of abnormal fractionated and/or late signals identified during sinus rhythm voltage mapping; B: Provides an example, as exhibited by the white arrows, of an isolated late potential. AP: Anterior.

ping and ablation procedures in patients with ARVC/D and VT have demonstrated the important role of the epicardium. Abnormal epicardial low-voltage areas are typically much larger than the corresponding endocardial region; with extensive networks of late activation and fractionated signals^[10,16]. Assessment of the epicardial voltage map should be performed with voltage threshold set to 1.0 mV to identify abnormalities consistent with scar as opposed to epicardial fat (Figure 3)^[17]. Due to the widespread extent of confluent scarring in these patients, it is very common to identify multiple VT circuits that may involve both endocardial and epicardial surfaces. In addition, the dense mid-myocardial/sub epicardial fibrosis can create an effective barrier for endocardial to epicardial spread of activation. The resultant layered and delayed activation of the epicardium from the edges of the scar creates the milieu for an isolated VT circuit entirely confined to the epicardium and requiring epicardial access and direct ablation for elimination (Figure 4)^[18]. In patients that have failed endocardial ablation, repeat ablation targeting the epicardial circuits was associated with superior long-term success rates^[10]. For these reasons, the operator should always anticipate a high likelihood of needing epicardial access for mapping and ablation to achieve a successful outcome.

Although identification of abnormal epicardial substrate is best achieved through a percutaneous pericardial puncture, analysis of unipolar endocardial voltage maps with the associated larger field-of-view, provides information pertaining to the degree of epicardial abnormality present. Areas of unipolar voltage < 5.5 mV are associated with epicardial abnormalities. Unipolar voltage abnormalities identified during RV endocardial mapping that far exceed the bipolar endocardial substrate is highly suggestive of a more extensive epicardial $>$ endocardial substrate that is consistent with the ARVC/D substrate in patients with VT (Figure 5). Additional clues to the requirement for epicardial mapping and ablation include surface ECG morphologies of VT suggesting epicardial exits (QS complex in the inferior leads and/or right pre-

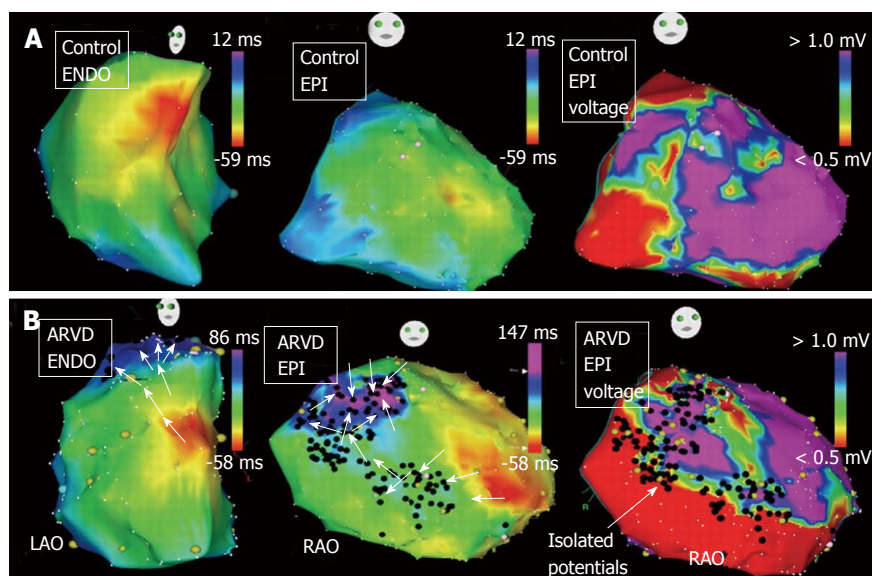


Figure 4 Right ventricle endocardial and epicardial activation maps and epicardial bipolar voltage maps of a control patient (A) and a patient with arrhythmogenic right ventricular cardiomyopathy/dysplasia (B) are shown. A: From control patient demonstrates continuous and rapid activation from the anteroseptal region toward the infundibulum and tricuspid annulus. The endocardial (not shown) and epicardial voltage map did not reveal any late potential or substantive voltage abnormalities; B: From patient with ARVC/D demonstrates significant epicardial scarring with epiendo isolated late potentials (black tags) on the bipolar voltage map. The activation wavefront is significantly delayed into the scar due to the extensive epicardial disease. Adapted from Haqqani *et al*^[18] with permission. RAO: Right anterior oblique; LAO: Left anterior oblique.

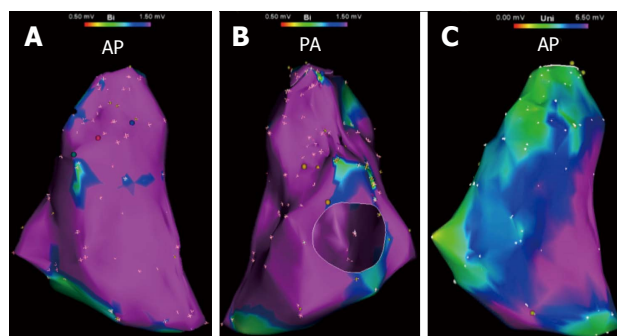


Figure 5 Bipolar and unipolar endocardial right ventricle voltage maps in a patient with ventricular tachycardia in the setting of arrhythmogenic right ventricular cardiomyopathy/dysplasia. A and B: Demonstrate no substantial endocardial substrate on bipolar voltage map; C: Demonstrates a substantial area of unipolar voltage abnormality (< 5.5 mV) encompassing most of the right ventricle free wall in the same patient. AP: Anterior; PA: Posterior.

cordial leads), the presence of isolated epicardial scar on magnetic resonance or intracardiac echo imaging, and/or prior failed endocardial ablation^[16,19,20].

Disease progression

Although much has been learned about the process of fibrosis underlying ARVC/D, there continues to be significant phenotypic variability for reasons that have not been clearly elucidated. Of note, multiple genes that have been implicated in the disease and this may lead to marked variability of phenotypic expression. It is unclear if disease progression is the result of a continuously progressive degenerative process or rather periods of disease stability followed by serial deteriorations associated with a distinct triggering event. The belief that ARVC/D is a

degenerative disorder has notably influenced treatment plans, particularly referral for catheter ablation. The degenerative hypothesis has been used as an explanation of unfavorable outcomes, thus labeling catheter ablation as a limited therapeutic option for patients with ARVC/D and VT. We have demonstrated, utilizing detailed electroanatomic mapping, progressive RV dilatation in patients presenting for repeat ablation procedures, but with no or only minimal macroscopic scar progression in a majority of patients (Figure 6)^[21]. This data along with the favorable outcome following endocardial/epicardial ablation and the demonstrated complex relationship between various genetic components and possible environmental or acquired factors favors a disease etiology that is not a primary deteriorating process.

PROCEDURAL APPROACH-LESSONS LEARNED

Mapping and ablation

Detailed assessment of the endocardial and epicardial electroanatomic maps has provided the much-needed insights into the complex abnormal substrate in patients with ARVC/D and VT. The cornerstone of developing a successful ablation approach in these patients requires a thorough understanding of this underlying substrate, particularly recognizing the importance of the epicardium. Through this evolving process, we have developed a systematic approach to evaluating the substrate and performing catheter ablation in these patients, much of which is centered on important lessons learned from within the electrophysiology laboratory over the last 2 decades.

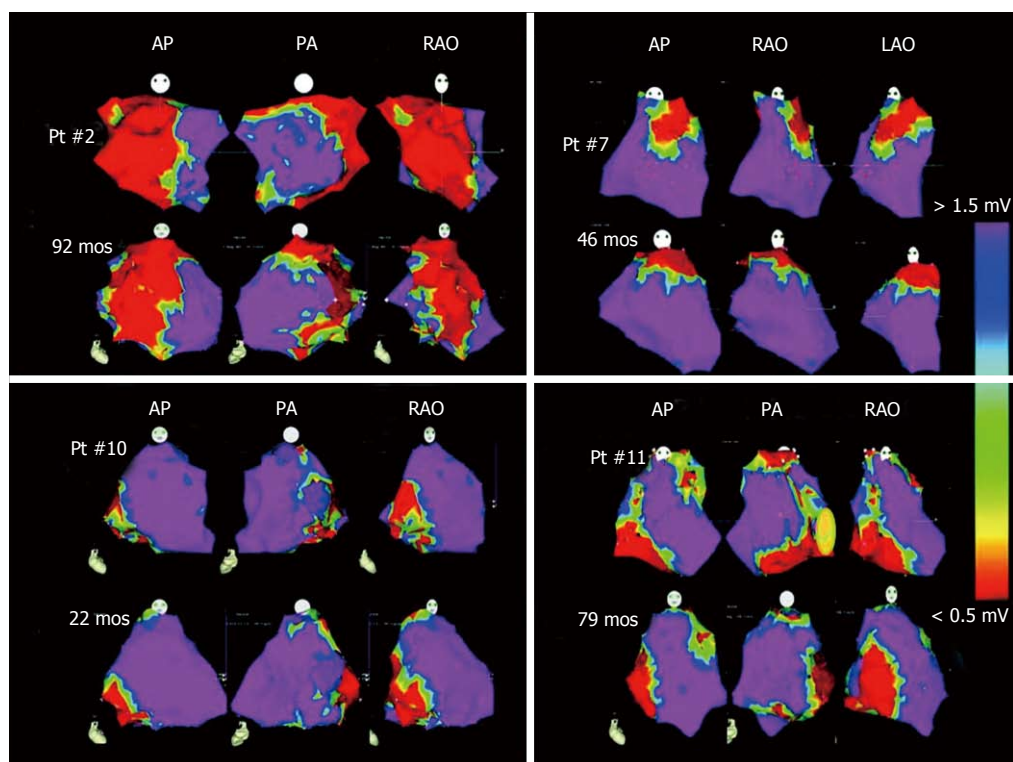


Figure 6 Right ventricle endocardial sinus rhythm bipolar voltage maps for 4 patients who did not develop scar progression over time. Each patient shows complementary views in the anterior (AP), right anterior oblique (RAO), and left anterior oblique (LAO) projections during initial and subsequent catheter ablation procedures. Normal voltage regions are represented in the purple regions and low voltage areas are represented in red. Adapted from Riley *et al.*^[21] with permission. PA: Posterior.

The general principles common to the ablation of all scar-related VT also apply in ARVC/D. However, in contrast to the post-infarction patient, the operator should anticipate a high likelihood of needing epicardial access for mapping and ablation to achieve a successful outcome for the reasons discussed. Evaluation in the EP laboratory typically begins with patients under conscious sedation to maximize the chances that induced VTs will be hemodynamically tolerated. A detailed RV endocardial voltage map is created in sinus rhythm using the standard 0.5-1.5 mV voltage cutoffs to define the endocardial substrate as previously discussed^[13,22]. Special attention is focused on the periannular area and any identified low-voltage areas to ensure adequate sampling has occurred^[23-25]. Occasionally, it can be technically challenging to perform catheter manipulation in the periannular tricuspid valve region. It is imperative to ensure adequate catheter contact during mapping to confirm low-voltage areas are from abnormal substrate and not inadequate catheter-tissue contact. This process can be facilitated by (1) using a sheath that extends transvenously to the tricuspid valve and provides stability; and/or (2) looping the mapping catheter in the RV to facilitate acquisition of detailed recording along the free wall adjacent to the annulus. Colored tags are placed on the electroanatomic map when fractionated signals and/or isolated late potentials are identified to keep track of their location^[26,27]. Pacemapping is performed at sites of interest with late potentials and other multicomponent electrograms and are carefully analyzed. A match of the

pacemap QRS morphology of the VT coupled with a long stimulus to QRS interval will identify additional sites of interest, which are given their own unique color tag. A sudden transition in paced QRS morphology coupled with changes in the stimulus to QRS interval may define anatomic boundaries of the isthmus or if a long stimulus to QRS is still identified a critical isthmus of conduction that will need to be tagged and ultimately targeted for ablation.

After completing the endocardial RV sinus rhythm substrate map and detailed pacemapping, programmed ventricular stimulation is performed. ICD electrograms are also recorded when VT is initiated. Induced VT ECG morphology and ICD electrograms are compared to previously captured clinical arrhythmias in addition to the pacemap morphologies that were obtained during sinus rhythm mapping. Assessment of ICD electrograms may be especially useful if clinical arrhythmia ECG tracings are unavailable^[28]. An endocardial ablation strategy is guided primarily by activation and entrainment mapping whenever possible of any hemodynamically tolerated VTs. It is not uncommon for unstable VT to be induced that is characterized by changes in morphology with any catheter manipulation or rates that results in hemodynamic instability. The VTs may not be amenable to localization utilizing conventional activation and entrainment mapping techniques. In these cases, ablation is guided by pacemapping and detailed substrate assessment. Ablation lesions are usually applied with an irrigated tip catheter

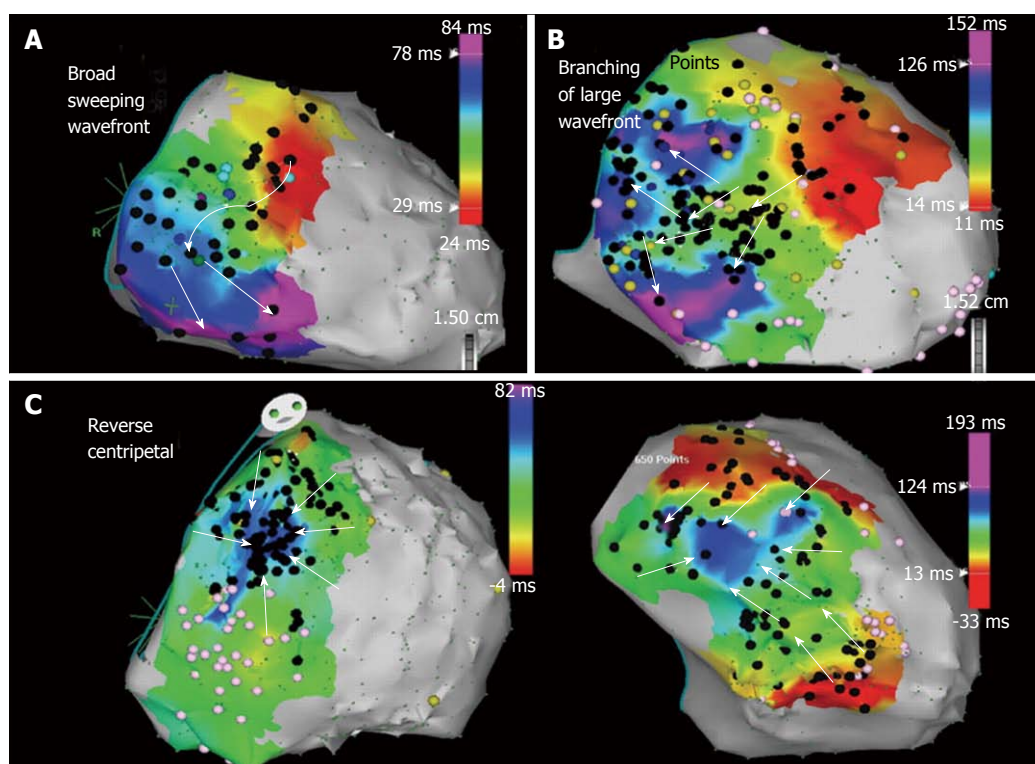


Figure 7 Epicardial right ventricle free wall activation maps illustrating propagation wavefront of epicardial isolated potentials in a patient with arrhythmogenic right ventricular cardiomyopathy/dysplasia. A: Right ventricle (RV) free wall activation via a broad wavefront progressing toward the inferior RV; B: A diverging pattern of activation, initially broad, but subsequently branching as it progresses through the scar; C: Reverse centripetal pattern with outside activation progressing inward with wavefront collision in the center of the scar. Adapted from Haqqani *et al*^[16] with permission.

for a minimum of 90 s. Power delivery begins at 20 Watts and is typically titrated to a maximum of 40 Watts to obtain a 12-15 Ohm impedance drop or approximately 10% decrease from the baseline impedance.

Any VT that can be mapped and successfully ablated from the endocardium is targeted initially. The use of irrigated RF energy delivery may eliminate all induced VTs with endocardial ablation alone^[13] though this is less likely in the context of ARVC/D than in ischemic VT. Epicardial mapping is required in cases when inducible VT is still present after endocardial ablation and should be planned for most patients.

When appropriate, the next step in the mapping and ablation procedure is to obtain intrapericardial access and perform epicardial mapping^[29]. We prefer to perform detailed endocardial mapping and ablation before proceeding with epicardial access. This has the advantage of eliminating many of the VTs and allowing for some VT control in most patients should the procedure be aborted due to difficulties or complications that may occur from the pericardial puncture.

Patients are usually placed under general anesthesia prior to performing the pericardial puncture, though we have performed the procedure under conscious sedation with remifentanyl and midazolam in select cases^[30]. A posterior access approach after subxiphoid entry with the Touhy needle is favored as patients with ARVC/D typically have RV dilatation that may increase the risk of RV perforation with an anterior approach (surgical backup

should always be readily available throughout the procedure). The posterior epicardial approach should begin just under the rib margin to minimize risk of liver laceration (Supplemental Video). After pericardial access is obtained, a detailed epicardial sinus rhythm voltage map is created in a similar fashion as described for the endocardium, but with voltage threshold set to 1.0 mV to identify abnormalities consistent with scar as previously described^[17]. Programmed stimulation is repeated and, if hemodynamically tolerated, the induced VTs are mapped using conventional activation and entrainment techniques. When this is not possible, as is frequently the case and further exacerbated by additional anesthetic vasodilatory effects, a substrate ablation strategy is required. An extensive epicardial lesion set is designed to incorporate sites of pacemap QRS matches to induced or clinical VT morphologies and all markedly abnormal multicomponent or late electrograms within the low voltage area that were identified during the detailed substrate map. On occasion we have been able to map the sequence of late potentials from earliest to latest originating at the scar border (Figures 4 and 7) and targeting the earliest late potential can effectively eliminate a large area of subsequent late potential activation. Occasionally, areas of abnormal electrograms can extend beyond the defined area of low voltage and these signals, late or split electrograms, should be targeted for ablation particularly if associated with a long stimulus to QRS and QRS morphology with pacing that matches the VT. These observations emphasized the crucial importance of pay-

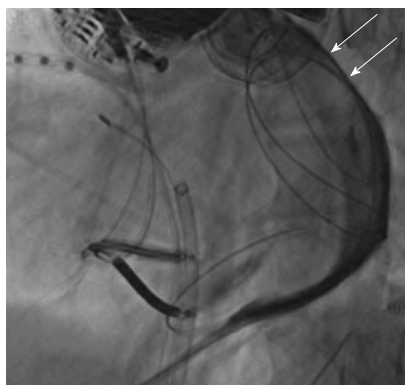


Figure 8 Fluoroscopic image in the left anterior oblique projection showing distribution of contrast restricted by pericardial compartmentalization from prior epicardial mapping and ablation. In this particular case, a deflectable catheter with a steerable sheath was not able to disrupt the adhesions and a more anterior epicardial access was required to bypass the area of compartmentalization to target the area of interest. Adapted from Tschabrunn *et al*^[33].

ing attention to electrogram characteristics as well as voltage when performing epicardial substrate mapping.

Special considerations pertaining to epicardial mapping and ablation

Coronary angiography is performed prior to epicardial ablation to ensure adequate distance between the coronary arteries (particularly the RV marginal branch of the right coronary artery) and ablation catheter. Phrenic nerve proximity to epicardial ablation sites is not an issue in ARVC/D cases although direct diaphragmatic stimulation may occur on the diaphragmatic surface of the RV. Epicardial ablation parameters for energy delivery are generally similar to the endocardium although the irrigation flow-rate is maintained at a lower rate (10-17 mL/min) to avoid unnecessary fluid accumulation with less concern about coagulum formation. Ideally, intracardiac echocardiography (ICE) is used to monitor lesion development and to assess for any complications throughout the procedure. Epicardial fat and thick fibrofatty replacement tissue can make it difficult to create lesions during epicardial ablation. It is important to ensure good contact at the catheter-tissue interface and optimal impedance drops for each lesion.

At the conclusion of the procedure, triamcinolone acetate (2 mg/kg) is injected into the pericardial space and allowed to disperse for 15 min before any suction is applied^[31]. This has been shown to minimize the severity of the pericardial inflammatory reaction following epicardial ablation lesion delivery and facilitates future percutaneous pericardial access if required^[32]. The pericardial drain is then attached to a passive suction device and removed the following morning after a transthoracic echocardiogram has confirmed no fluid accumulation overnight.

If a repeat procedure is required, the operator should be prepared to encounter pericardial adhesions during repeat epicardial mapping and ablation. These adhesions will be typically limited early after the initial procedure. The adhesions can usually be interrupted with the use

of a steerable flexed catheter tip coupled with a steerable sheath. Elimination of the adhesions will allow for detailed mapping^[32]. Careful monitoring for bleeding is important when disrupting the adhesions and is facilitated by utilizing ICE throughout this process. It is possible for dense adhesions to compartmentalize the epicardial surface and preclude access to the entire surface without re-accessing the pericardial space with a more anterior puncture (Figure 8)^[33]. When adhesions are found to be resistant to dissection, consideration should be made to have the patient undergo an open-chest procedure through a surgical incision, as the adhesions may not be safely lysed if a flexed catheter “u” shape cannot be advanced. A sternotomy may be required to permit dissection of matted pericardium that may not be amenable to catheter dissection.

Procedural endpoints

Our experience has consistently demonstrated that extensive endocardial and epicardial ablation is likely required to abolish all induced VTs. The primary procedure endpoint in these cases is both the elimination of a majority of abnormal electrograms and elimination of all induced VTs. Aggressive programmed stimulation should be performed as part of the evaluation of efficacy, including 2 stimulation sites, the introduction of up to triple extrastimuli, and the use of isoproterenol before the VT is described as non-inducible.

Non-invasive programmed stimulation through the ICD is performed 1-2 d after the ablation off antiarrhythmic drugs (AADs) to ensure continued non-inducibility^[34]. Induction of VT suggests the need for further ablation in order to achieve a favorable long-term antiarrhythmic drug-free outcome.

Whenever possible, AADs are discontinued and patients continue with beta-blocker therapy only. Approximately one third of our patients continue to be treated with low dose sotalol therapy, but the effort to discontinue amiodarone has been successful in all but one patient in our experience with 62 ARVC patients.

CONCLUSION

Ventricular tachycardia in ARVC/D patients can be a difficult clinical problem to manage. Antiarrhythmic medications are often ineffective or not tolerated leaving these young and active patients at high risk for recurrent ICD shocks. Much has been learned about the underlying arrhythmia substrate and the appropriate strategies required to facilitate successful catheter ablation. This comprehensive and extensive ablation strategy that targets both the endocardial and epicardial substrate with elimination of abnormal electrograms offers long-term, drug-free arrhythmia control in a majority of patients.

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Angiotensin II-related hypertension and eye diseases

Pablo Jesus Marin Garcia, Maria Encarna Marin-Castaño

Pablo Jesus Marin Garcia, Maria Encarna Marin-Castaño, Department of Ophthalmology, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL 33136, United States

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Correspondence to: Maria Encarna Marin-Castaño, MD, PhD, Department of Ophthalmology, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, McKnight Bld, 1638 N.W. 10th Avenue, Miami, FL 33136, United States. mcastano@med.miami.edu

Telephone: +1-305-4825142 Fax: +1-305-4825095

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Core tip: Association between eye diseases and systemic hypertension has been revealed. The developments of some ocular diseases, as well as, alterations in the severity of these diseases have been associated with dysregulation of the ocular renin-angiotensin system and activation of the angiotensin type 1 receptor. In this paper we reviewed the importance of angiotensin II in the etiology of age-related macular degeneration and diabetic retinopathy, two ocular diseases that can rob people of their vision.

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Abstract

Systemic vascular disease, especially hypertension, has been suspected as a risk factor for some eye diseases including, diabetic retinopathy and age-related macular degeneration. Hypertension can contribute to chronic diseases by hemodynamic injury and/or cellular actions induced by hypertension-related hormones or growth factors. Among the most important is Angiotensin II (Ang II), which controls blood pressure and induces different cellular functions that may be dependent or independent of its effect on blood pressure. Importantly, as is true for heart, kidney and other organs, the renin-angiotensin system (RAS) is present in the eye. So, even in the absence of hypertension, local production of Ang II could be involved in eye diseases. The goal of this manuscript is to review the most relevant scientific evidence supporting the role of the RAS activation, in the development of age-related macular degeneration and diabetic retinopathy, and highlight the importance of Ang II in the etiology of these diseases.

INTRODUCTION

Knowledge of the renin-angiotensin system (RAS) has advanced remarkably over recent years from that of a classical endocrine system that explained homeostasis for maintenance of circulating intravascular volume and thereby restoration of arterial pressure to a newer concept including a number of local RASs that operate independently within several organs^[1-5], including the eye^[6,7].

Angiotensin II (Ang II), a hormone that raises blood pressure, is derived either from the circulation or from local production. Ang II causes vasoconstriction, sympathetic nervous stimulation, release of aldosterone, and renal actions which contribute to control the blood pressure^[8]. The effects of Ang II provoke different responses in tissue, which are mostly mediated *via* the Ang II type 1 receptor (AT1R). According to previous studies, the systemic RAS is not supposed to be directly accountable for the increase in blood pressure, it appears to be that

Table 1 Presence of renin-angiotensin system components in the eye

RAS molecule	Eye part	Species	Ref.
Prorenin	Retina	Human	Sramek <i>et al</i> ^[207] , 1988
	Ciliary body	Human	Danser <i>et al</i> ^[33] , 1989
	Vitreous body	Human	Danser <i>et al</i> ^[33] , 1989
Retina	Retina	Human, rabbit	Danser <i>et al</i> ^[33] , 1989
	Ciliary body	Rabbit	Wagner <i>et al</i> ^[19] , 1996
	Choroid	Human, Rabbit	Ramirez <i>et al</i> ^[208] , 1996
	Iris	Rabbit	Ramirez <i>et al</i> ^[208] , 1996
	Vitreous	Human, rabbit	Ramirez <i>et al</i> ^[208] , 1996
	Aqueous humor	Rabbit	Ramirez <i>et al</i> ^[208] , 1996
	Retina	Human, rabbit	Sramek <i>et al</i> ^[209] , 1992
Angiotensinogen	Ciliary body	Human, rabbit	Ramirez <i>et al</i> ^[208] , 1996
	Choroid	Human, rabbit	Wagner <i>et al</i> ^[19] , 1996
	Iris	Human, rabbit	Wagner <i>et al</i> ^[19] , 1996
	Vitreous	Human, rabbit	Wagner <i>et al</i> ^[19] , 1996
	Aqueous humor	Rabbit	
	Retina	Dog, monkey, human	Vita <i>et al</i> ^[210] , 1981
	Ciliary body	Rabbit, porcine	Weinreb <i>et al</i> ^[211] , 1985
ACE1	Choroid	Human, rabbit, porcine	Immonen <i>et al</i> ^[212] , 1987
		Dog, monkey, human	Ramirez <i>et al</i> ^[208] , 1996
		Rabbit, porcine	Wagner <i>et al</i> ^[19] , 1996
	Sclera	Dog, monkey	Shiota <i>et al</i> ^[213] , 1997
	Iris	Rabbit, porcine	Geng <i>et al</i> ^[214] , 2003
	Cornea	Human	Savaskan <i>et al</i> ^[16] , 2004
	Vitreous	Dog, monkey, rabbit	Savaskan <i>et al</i> ^[16] , 2004
	Aqueous humor	Human, dog, monkey, rabbit	Savaskan <i>et al</i> ^[16] , 2004
	Tear fluid	Human, rabbit	Savaskan <i>et al</i> ^[16] , 2004
	Retina	Rodent	Tikellis <i>et al</i> ^[215] , 2004
		Human	Senanayake <i>et al</i> ^[17] , 2007
ACE2			
Chymase	Choroid	Dog	Shiota <i>et al</i> ^[213] , 1997
	Sclera	Dog	Maruichi <i>et al</i> ^[216] , 2004
	Vitreous body	Human	
AT1R	Retina	Human	Savaskan <i>et al</i> ^[16] , 2004
	Cornea	Human	Senanayake <i>et al</i> ^[17] , 2007
	RPE	Human	Striker <i>et al</i> ^[18] , 2008
AT2R		Rodent	Praddaude <i>et al</i> ^[104] , 2009
	Retina	Human	Senanayake <i>et al</i> ^[17] , 2007
	RPE	Human	Striker <i>et al</i> ^[18] , 2008
		Rodent	Praddaude <i>et al</i> ^[104] , 2009
Ang I	Retina	Porcine	Danser <i>et al</i> ^[22] , 1994
	Choroid	Porcine	
	Vitreous body	Porcine, human	
Ang II	Aqueous humor	Human	
	Retina	Human, porcine, rabbit	Danser <i>et al</i> ^[22] , 1994
	Ciliary body	Human, rabbit	Ramirez <i>et al</i> ^[208] , 1996
	Choroid	Porcine, human, rabbit	Savaskan <i>et al</i> ^[16] , 2004
	Iris	Rabbit	Senanayake <i>et al</i> ^[17] , 2007
	Cornea	Human	
	Vitreous body	Porcine, human, rabbit	
	Aqueous humor	Human, rabbit	
Ang 1-7	RPE	Rodent	Praddaude <i>et al</i> ^[104] , 2009
	Retina	Human	Senanayake <i>et al</i> ^[17] , 2007

Ang: Angiotensin; RAS: Renin-angiotensin system; AT1R: Angiotensin II type 1 receptor; ACE1: Angiotensin-converting-enzyme 1.

the blood pressure and local blood flow (BF) adjustment are due to the local RAS^[9]. Ang II directly or indirectly also promotes apoptosis, hypertrophy, neovascularization, inflammation and fibrosis *via* AT1R activation^[10-13].

Ophthalmic literature concerning the RAS started in 1977 with a study by Igić *et al*^[14] on the detection of angiotensin-converting-enzyme (ACE) activity in homogenates of the retina. Since then, and as shown in Table 1, the presence of all constituent of the RAS has been confirmed in different parts of the eye (Figure 1), where

the mediators of the RAS are locally released, conferring the molecular basis for a biological function of these mediators in the eye^[15-18]. However, the origin of intraocular mediators such as Ang II and renin has been debated. Local synthesis of both renin and ACE has been suggested in the retina of rats^[19]. In this way, the secretion of renin by retinal pigment epithelium (RPE) to the retinal side was demonstrated by Milenkovic *et al*^[20] (2010). It has been also suggested that Ang I, Ang II, and angiotensinogen are not able to cross the barriers between eye

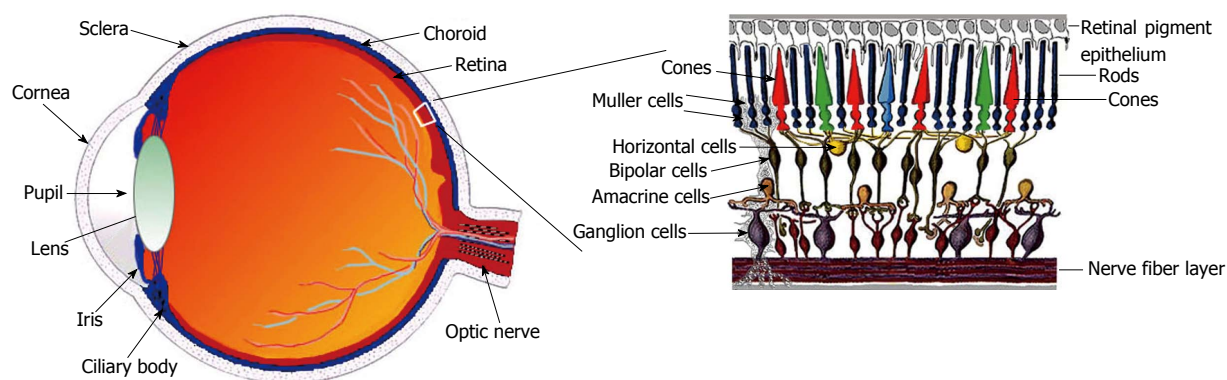


Figure 1 A drawing of a section through the human eye with a schematic enlargement of the retina [Helga Kolb from AMER Sci (2003)].

and circulating blood^[21,22]. On the other hand, the presence of an ocular local production of Ang II has been indicated^[22,23]. As a result, increased local or tissue Ang II formation in the retina in the absence of elevated circulating Ang II may indeed be deleterious.

The RPE, a cell layer between the neurosensory retina and choroid, nourishes retinal visual cells and forms part of the blood-retinal barrier, therefore, playing a central role in maintaining retinal function. For example, the presence of the AT1R in the RPE basolateral membrane^[20], indicates that the systemic RAS is a part of that retinal function signaling. Interestingly, by using electroretinography, it was previously demonstrated that regulation of the systemic RAS changes the neuro-sensory retina activity^[24-26]. Furthermore, plasma Ang II cannot pass into the eye^[7], and modifications of the renin expression in the RPE by regulators of the systemic RAS alter, have been observed^[24]. Overall, these data lead to think the systemic RAS credits the presence of an intraocular RAS through the RPE.

The presence of the most important RAS components in the retina and the Ang II actions observed in the eye (Surveying PubMed for eye, ocular, or retina, and Ang yields 734 citations dating back to 1963), imply an important role of RAS in the eye. However, its exact role, remains inadequately recognized. Of special focus are the components of the RAS and its receptors in the retina, as the RAS is increasingly recognized as a mediator of the pathogenesis of ocular diseases such as age-related macular degeneration (AMD) and diabetic retinopathy (DR)^[27-36], which are two major causes of severe vision loss and blindness. Therefore, in this manuscript we review the most relevant scientific evidence supporting the function of the RAS activation, in the development of AMD and DR, and highlight the importance of Ang II in the etiology of these two ocular diseases.

RETINAL MICROVASCULATURE: MODULATION BY ANG II

Given that vascular pathology in the retina is an important contributor of vision loss, the greatest research examining retinopathy and the possible role played by the RAS

has been focused on the microvasculature. The circulatory system of the retina supplies oxygen and nutrients to retinal tissue, which is essential for a correct function.

The retina circulation essentially comprises two parts: (1) a retinal circulation without autonomic innervation; and (2) a choroidal vasculature with autonomic innervations^[37]. Evidence is accumulating that the retinal microvasculature is an interactive complex that includes a network of capillaries and a tertiary arteriole that links the capillaries with a secondary arteriole (Figure 2). The capillary is formed by an uninterrupted endothelium and inner pericytes^[38]. Both endothelial cells and pericytes are directly communicated and share a common basement membrane^[39]. It was previously demonstrated that contraction and relaxation of pericytes leads to alterations in the capillary lumen, which could regulate local perfusion^[40-45]. Moreover, evidence suggests that a capillary network including pre-capillary at the tertiary arteriole form a working unit which is able to control local perfusion within the retinal vessels^[39,46,47].

The retina tends to keep its BF constant through an autoregulatory response that is intrinsic^[48,49]. The autoregulation of the retinal microcirculation is evaluated by some methods, including changes in systemic blood pressure^[50]. The main regulators of BF are the vascular pericytes^[51,52], endothelium cells and the neural and glial cells^[53]. One of the most important peptides playing a crucial role in the regulation of vasculature tone is Ang II^[54-58]. For instance, it has been demonstrated that Ang II induces retinal endothelial cells apoptosis^[59] and constriction of pericytes^[60-63], therefore, decreasing the mean retinal arterioles and capillaries diameter, which leads to BF reduction^[51,52].

Modifications in the retinal BF has been observed in some eye disorders. For example disturbances in the ocular circulation have been reported in AMD^[31-33], supporting the presence of hemodynamic abnormalities in this disease. AMD is the main cause of severe visual loss and legal blindness in elderly. There are three stages of AMD: (1) early AMD, which is diagnosed by the presence of medium-sized drusen; (2) intermediate AMD, characterized by the presence of large drusen and/or pigment changes in the retina; and (3) late AMD, in which in addition to drusen, there is damage of the macula with severe

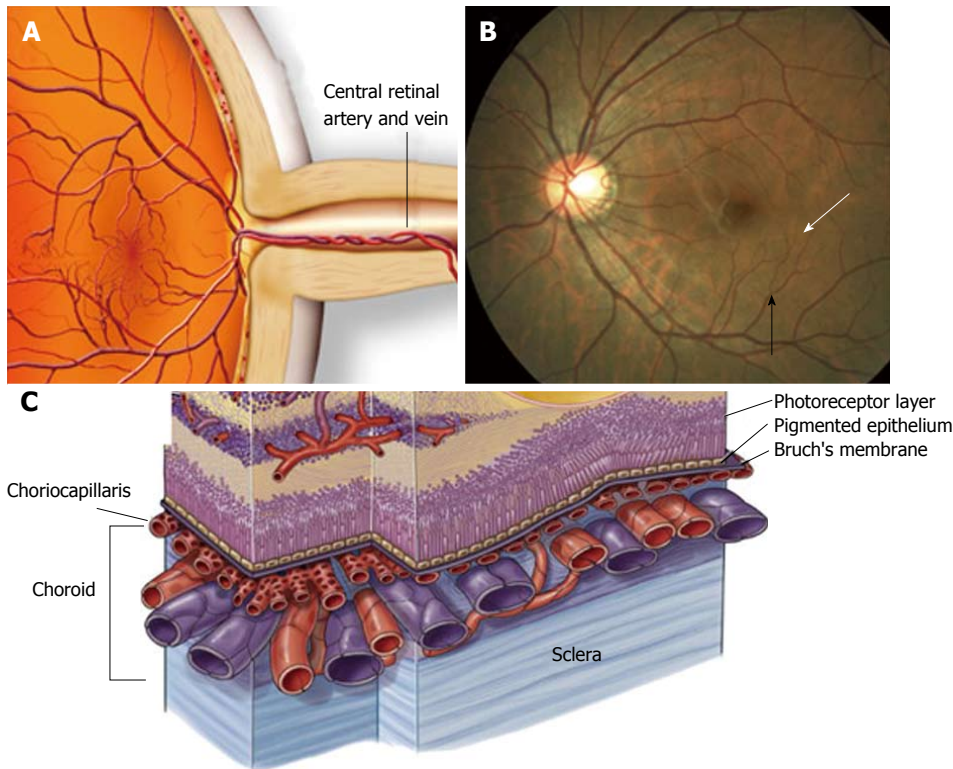


Figure 2 Anatomy of ocular circulation. A: Central retinal artery and vein respectively; B: Arteriole (black arrowhead); capillaries (white arrowhead); C: Choroidal vasculature (Anand-Apte, Hollyfield, Academic Press, Elsevier Books, 2009; 9-15).

vision loss^[64]. Both local ocular and systemic vascular risk factors, such as systemic hypertension seem to be connected with the etiology of AMD. A relationship between AMD and modifications in the eye circulation was previously reported^[27,29,65-72] and numerous studies have proposed a decrease in the vascularity of the choroid^[73-75], reinforcing the existence of hemodynamic abnormalities in this disease. The relationship between impaired choroidal perfusion, reduced choroidal BF and clinical manifestations of AMD has been recently reported by previous studies^[70,71,75-79].

Association between AMD and systemic hypertension has been studied by many epidemiological studies^[80-84]. The Macular Photocoagulation Study has demonstrated that patients with both, AMD and hypertension responded less to laser photocoagulation treatment than patients with only AMD^[85]. These observations, suggested that hypertension could have a harmful effect on the stages of AMD. A decrease in the choroidal BF in individuals with hypertension versus those without was previously reported^[31,32]. These authors, also showed that this reduction becomes more marked with increasing AMD severity^[31,32]. Therefore, the observed decrease in choroidal BF in AMD patients with hypertension suggests the implication of an ischemic mechanism in the etiology of AMD.

ANG II -RELATED HYPERTENSION IN THE PATHOGENESIS OF AMD

AMD is a slow progressing disease that can rob people

of their vision. This ocular disease is a public health problem that will remain a major threat to vision.

There are two forms of AMD; early (dry) AMD and late (wet) form. Wet AMD is always preceded by early disease, and in about one-third of cases dry AMD can lead to wet macular degeneration which progresses much more rapidly and leads to greater loss of central vision. Death of photoreceptors is the ultimate cause of vision loss. However, the initial cellular target of this disease is the RPE, its extracellular matrix, and the subjacent vascular bed (called choriocapillaris; Figure 2C), the blood supply for the outer retina.

Dry AMD is characterized by the accumulation of debris and other lipid rich extracellular deposits in form of drusen under the RPE and within Bruch's membrane (BrM) (Figure 3B)^[86,87]. During aging, deposits initially accumulate between the RPE and its basement membrane (called BLD), but progression into AMD requires additional deposit formation within BrM, (called BLiD and "nodular" drusen). These are yellowish lesions that can be seen in the macula at the earliest stages of dry AMD. A finding in dry AMD that represents disease progression and can be used as a surrogate endpoint is the presence, size, and appearance of drusen. Over time, these drusen enlarge, coalesce, become pigmented, and eventually can disappear when they progress to the late form of AMD. We observed that when drusen go away, there are three possible outcomes; formation of geographic atrophy, formation of abnormal blood vessels known as wet AMD or choroidal neovascularization (CNV) (Figure 3C), or disappearance of drusen without any significant

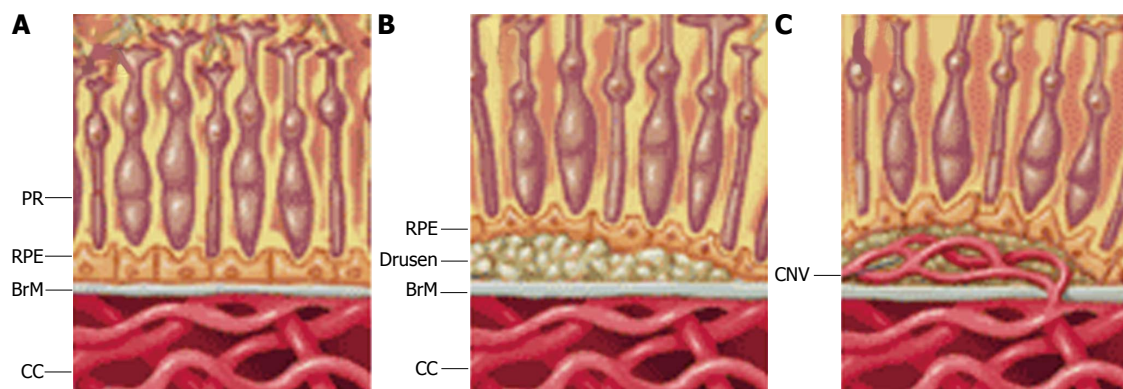


Figure 3 The pathologic changes to the retina and choroidal blood vessels typical of dry and early wet age-related macular degeneration respectively. A: Control; B: Early age-related macular degeneration (AMD); C: Wet AMD. PR: Photoreceptors; RPE: Retinal pigment epithelium; BrM: Bruch's membrane; CC: Choriocapillaries; CNV: Choroidal neovascularization (provided by the OcuCure Therapeutics' website).

anatomic abnormality. The endpoint that represents the progression of the disease is the growth and enlargement rate of drusen^[88-90]. Wet AMD is always preceded by early disease.

Our understanding of this disease has increased; however, no one knows exactly what causes AMD. Age is the major factor determinant for developing AMD. However, it has been suggested that the disease results from some interactions between different issues: genetic susceptibility, environmental factors and systemic health co-factors^[91-95]. Because the increasing frequency of hypertension, the RAS is of special interest among these systemic health co-factors. In this context, epidemiological demonstrated an association between hypertension and incidence of drusen^[28] and with wet AMD development^[29,96-98]. Exciting findings which showed a strong link between hypertension and progression of early AMD to the wet form were recently published^[99]. However, the mechanism(s) by which hypertension contribute to the progression from early form to CNV was not elucidated. In recent years, evidence has revealed that Ang II, AT1R signaling, and prorenin, may play a significant role in the mentioned pathologic processes^[100-104]. Moreover, recent studies revealed the participation of AT2R, Ang I and Ang 1-7^[24]. Consequently, investigation of the local RAS in the retina will allow find out new approach for the development of new treatments.

Dry (early) AMD

As mentioned previously, RPE-derived debris and other debris accumulated between the RPE and within BrM is a very well-known histopathologic sign of the dry AMD^[105-108]. Studies in eyes from AMD patient found out deposits of RPE-derived debris within BrM^[109]. Nevertheless, the mechanism(s) by which the debris accumulate were not studied. Based in the idea that a relationship between matrix metalloproteinases (MMPs) and inhibitors of matrix metalloproteinases and development of dry AMD exists. We proposed that the evolution of the sub-RPE deposits into BrM necessitates breakdown of the RPE basement membrane's components by diges-

tion or degradation of these compounds (*i.e.*, type IV and I collagens and laminin)^[110,111], and that ECM turnover up-regulation through activation of MMP-2 and MMP-14 is required for the interruption of these physical barriers. We evaluated the regulatory effects of Ang II and prorenin-activated prorenin receptor (PRR) on the MMP-2 and basement membrane component proteins, in the RPE. The objective of our work was to describe the expression and function of Ang II receptors in the RPE and at explore the contribution of this hormone and PRR in the etiology of dry AMD. Mice were rendered hypertensive either by exogenous administration of Ang II or by using a model of experimental renovascular hypertension (1K1C). Measurements of systolic blood pressure (BP) revealed a progressive increase during Ang II infusion period reaching a peak value on day 14 and remaining at plateau through day 30. However, after 24 h of exposure to Ang II, BP was not modified. Similarly, BP was significantly higher in 1K1C mice compared with the corresponding sham-operated group. No significant differences in BP were observed between control and sham-operated groups. Treatment using Ang II in combination with angiotensin receptors blockers showed that the AT1R blocker eliminated the modifications in the BP due to Ang II. However, the AT2R blocker did not alter the effect of Ang II on systolic BP, demonstrating, that the effect on BP caused by Ang II was AT1R mediated^[104,112].

Our study in human and mouse also confirmed that both ATRs were expressed and upregulated by Ang II in the RPE and showed that the activation of the AT1R by Ang II increased the intracellular calcium levels^[18,105]. These results clearly evidenced the functionality of the RPE's AT1R, which could be coupled to the phospholipase C-pathway. In contrast, activation of the AT2R by Ang II did not mobilize intracellular calcium. AT2R could be coupled to the cytosolic phospholipase A2 and not to the PLC pathway as shown for other tissues^[113]. Consequently, regulation of the AT2R transduction pathway is a possibility to be explored.

Ang II also up-regulated the activity of MMP-2,

MMP-14, and basigin (also known as extracellular matrix metalloproteinase inducer or cluster of differentiation 147) as well as digestion of type IV collagen^[18,104,112]. The Ang II observed effects were blocked by the AT1R antagonist candesartan. *In vivo*, the Ang II-derived decrease in collagen IV was AT1R/AT2R mediated, implying a synergistic effect. Therefore, Ang II through MMP-2, MMP-14, and basigin regulation could stimulate RPE basement membrane breakdown allowing the migration of BLD and buildup of BLiD deposits or drusen.

It is important to note that the majority of intracellular effects of Ang II in most tissues are MAPKs mediated. MAPKs are a group of serine/threonine kinases^[114-116] which can be divided into three major groups: ERK, p38, and Jun N-terminus kinase (JNK) and participate in a wide array of cellular responses including proliferation, differentiation, migration, and stress responses among others^[117-120]. We explored the involvement of MAPK as intracellular modulator of Ang II-induced up-regulation of MMPs in the RPE. Our study showed that Ang II-induced increase in MMP-2 activity is mediated by Erk(1/2) and p38 MAPK in the human RPE cell line ARPE-19. We also demonstrate that Ang II increased the expression of MMP-14, MMP-2 activity major regulator, in an Erk(1/2) and p38 MAPK-dependent way while basigin does not appear to be involved in RPE cells. In addition, we reported that Erk/p38 MAP kinase signaling pathway is AT1R mediated, which could be an important mechanism by which Ang II up-regulates MMPs in RPE cells. Moreover, we show that RPE from mice exposed to Ang II for 4 wk showed increased MMP-14 and basigin protein expression as well as increased phosphorylated Erk(1/2), p38, and JNK MAPK. The increase in MMP-14 protein expression and activation of Erk(1/2), p38, and JNK MAPK were AT1 receptor-mediated, whereas the increase in basigin expression increase was mediated by AT2 receptor^[112]. Blockade of extracellular signal-regulated kinases or p38 MAPK abolished the up-regulation of MMPs in RPE cells^[112]. Given that MMP-14 and basigin are major inducers of MMP-2, our results lead us to speculate that MMP-14 and basigin might regulate Ang II-induced MMP-2 activity through MAPKS- and AT1 receptor-dependent signaling pathways in the RPE. These original observations highlight the potential importance of this signaling pathway as a potential mediator of RPE response to Ang II-induced ECM dysregulation and disruption of the RPE basement membrane believed to be involved in sub-RPE deposits progression in the pathogenesis of AMD. Based on our observations, MAPKs inhibitors and AT1R blockers may prevent these changes in the ECM, which are essential in the development of early AMD.

We also provided evidence that activation of the PRR may be involved in ECM-remodeling through increase of collagen I^[121]. Interestingly, we confirmed that PRR and type I collagen were present in human retinas and that the expression of both proteins was higher in the RPE from dry AMD hypertensive donors (Figure 4), support-

ing our *in vitro* findings. Overall, our studies suggest a molecular mechanism by which hypertension may aggravate the pathology of dry AMD.

Even though dry AMD is not a retinal vascular pathology, we reviewed this form of the disease here because hypertension-related Ang II has been implicated in dry AMD pathogenesis^[28], and wet AMD is always preceded by the early form of the disease.

Wet AMD

As mentioned previously, about one-third of cases dry AMD can lead to wet macular degeneration which progresses much more rapidly and leads to loss of central vision. CNV is a retinal vasculature related pathology^[120] associated with several common retinal degenerative or inflammatory diseases^[87,120,122,123]. Inflammation and hypoxia are key cellular processes involved in the development of CNV^[17-25], in that choroidal monocytes processes, for example, have been noted to insert into BrM deposits suggesting that these sub-RPE deposits may generate inflammatory stimulus at the BrM and sub-RPE space. Macrophage infiltration to the damaged sites by chemotactic factors may be responsible for the production of inflammatory cytokines and angiogenic factors such as intercellular adhesion molecule 1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1)^[124] and vascular endothelial growth factor (VEGF)^[125] which will ultimately contribute to induction and/or progression of CNV^[26-28]. Blockade of AT1R by systemic administration of telmisartan reduced CNV formation, macrophage infiltration and expression of VEGF, VEGF receptor-2 (VEGFR-1), ICAM-1 MCP-1 and interleukin 6 in eyes from a laser induced CNV mouse model of AMD^[125]. This suggests that AT1R mediated up-regulation of these molecules and mediators participate in the development of CNV.

Ang II has been shown to act as an indirect mitogenic agent for retinal vascular endothelial cells by increasing VEGFR-2 expression^[23] which could lead to formation of CNV. Blockade of AT1R signaling suppresses pathologic but not normal retinal neovascularization by inhibiting inflammatory processes^[34,116]. Additionally, it has been shown that excised choroidal neovascular membranes from patients with AMD express AT1R, AT2R and Ang II on the vascular endothelium^[126]. Similar findings were seen in the laser-induced mouse model of CNV^[126]. As noted above, formation of CNV was suppressed with the AT1R blocker telmisartan but not with an AT2R antagonist^[127]. In a laser induced model of CNV using AT1R knockout mice, the ACE inhibitor, imidapril, significantly reduced choroidal and retinal neovascularization in wild type mice to levels detected in laser treated AT1R KO mice^[128]. Additionally, in a rat model of laser-induced CNV, losartan was shown to inhibit the incidence of new vessel formation from 99.5% to 72.5%^[129].

Increasing evidence support the notion that increase in the production of chemokines happens in diseases related to an inflammatory component. Several of these chemokines are expressed in the RPE cells, including

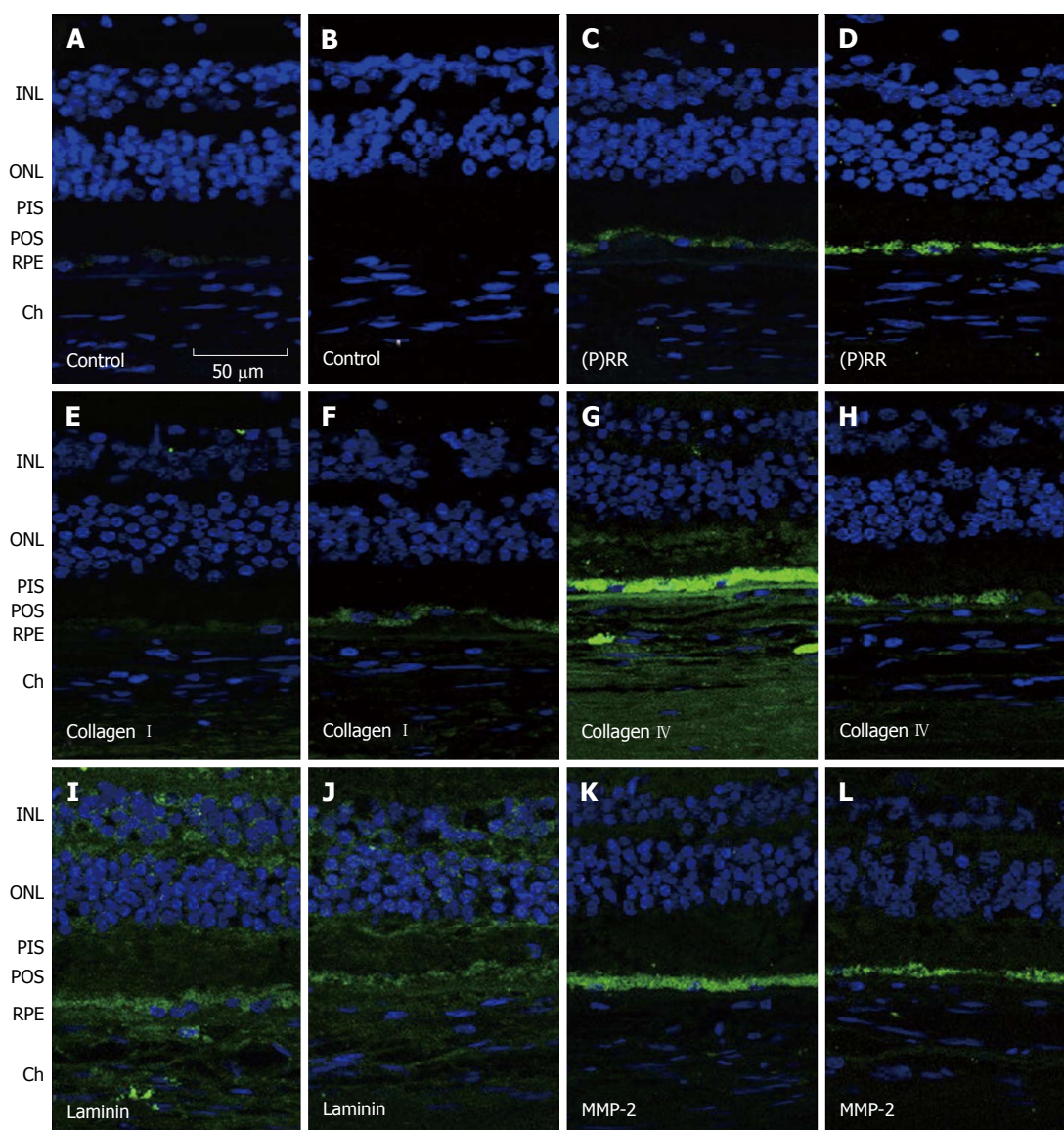


Figure 4 Representative immunofluorescent double staining of prorenin receptor, collagen types I and IV, laminin and matrix metalloproteinase-2 (green) and nuclei (bleu) in retina sections from human donor eyes with no known eye disease (B, D, F, H, J and L), and human donor eyes with dry age-related macular degeneration and hypertension (A, C, E, G, I and K)^[121]. Negative controls were generated by omission of the primary antibody (A and B). Sections were analyzed by using confocal microscopy (original magnification, $\times 40$). INL: Inner sections were analyzed with a confocal microscope at a magnification of $\times 40$. INL: Inner nuclear layer; ONL: Outer nuclear layer; MMP: Matrix metalloproteinase; PIS: Photoreceptor inner segments; POS: Photoreceptor outer segments; RPE: Retinal pigment epithelium; Ch: Choroid.

MCP-1^[29,30], which has been proposed to be implicated in the development of dry and wet AMD^[31-33]. During inflammatory responses, RPE cells have been shown to secrete MCP-1 toward the choroid, consequently, implying that RPE cells might induce recruitment of macrophage to the choroid^[34]. There is clear evidence for the role of MCP-1 in angiogenesis in several angiogenic-related disorders^[35-37]. Interestingly, expression of the recently discovered novel zinc finger protein MCP-1 induced protein (MCPIP) has been shown to induce tube formation in human umbilical vein endothelial cells^[38].

As mentioned previously, hypoxia, which was proposed to be one of the most significant driving forces for CNV formation^[130], is another key cellular process which stimulates the expression of VEGF in AMD. Angiogenic

factor expression occurring secondary to hypoxia is mediated by the family of transcription regulators known as hypoxia inducible factors (HIF). HIF-1 and -2 have been found to be expressed in human choroidal neovascular membranes^[131], and HIF-1 has been shown to upregulate expression of VEGF in RPE^[132,133]. Hypertension-associated Ang II is known to induce inflammation, macrophage infiltration, and angiogenesis by stimulating expression of MCP-1, HIF-1 and VEGF through the AT1R^[126,134-137]. Up-regulation of MCP-1 has been demonstrated in hypoxic animals^[138] and recently, it has been demonstrated that MCP-1 promotes angiogenesis *via* MCPIP, HIF-1 and VEGF induction^[139]. Interestingly, previous works also suggest that the BF in the choroidal and retinal is down-regulated in AMD hypertensive

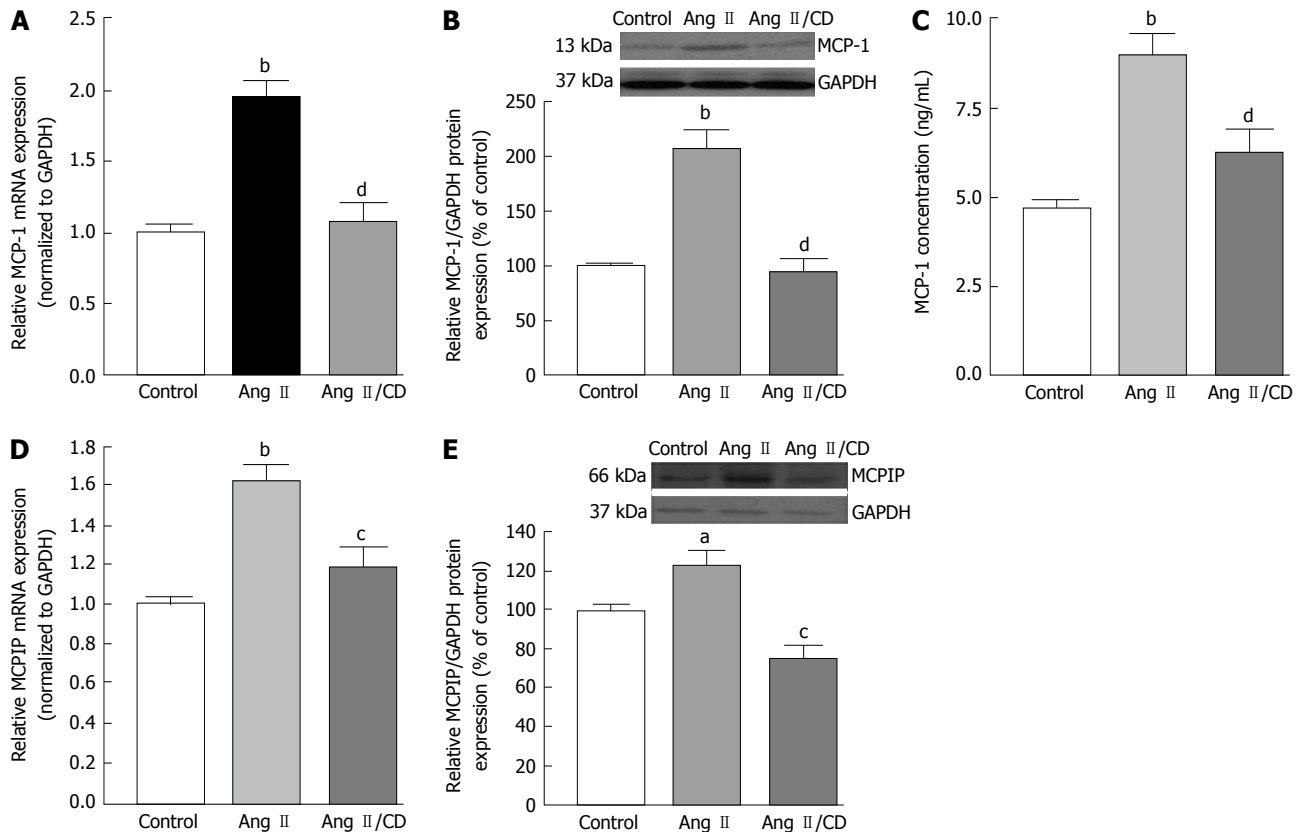


Figure 5 Hypertension-induced Angiotensin II up-regulated monocyte chemoattractant protein-1 and monocyte chemoattractant protein-1 induced protein expression through AT1R activation in retinal pigmented epithelium-choroid^[140]. C57BL 6 mice were treated with saline (1), Ang II (2), and Ang II in combination with candesartan (10 mg/kg per day) (3). Blood pressure was recorded before and after treatment. After 30 d of treatment, animals were sacrificed and eyes enucleated and collected for microdissection of retinal pigmented epithelium-choroid. Monocyte chemoattractant protein-1 (MCP-1) and MCP-1 induced protein (MCP-1P) proteins were analyzed by real-time PCR and Western blot. MCP-1 and MCP-1P mRNA expression by real-time PCR (A and D), protein expression by Western blot (B and E), and MCP-1 protein secretion by ELISA (C). GAPDH was used as control. Data are expressed as mean \pm SE ($n = 3$). ^a $P < 0.05$, ^b $P < 0.01$ vs control; ^c $P < 0.05$, ^d $P < 0.01$ vs Ang II-treated animals. CD: Candesartan. Ang: Angiotensin; PCR: Polymerase chain reaction; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; ELISA: Enzyme-linked immuno assay.

patients^[31,32], which leads to think about the possibility that an ischaemic/hypoxia mechanism plays a role in the CNV development. Given that a positive correlation between elevated levels of circulating MCP-1 and hypertension has been previously shown, we studied whether hypertension-induced Ang II influences the development of CNV and characterized the role played by MCP-1/MCP-1P in this event. We addressed this by setting goals of understanding the mechanisms underlying the interactions between the RPE, choroidal microvascular endothelial cells (cEC) and Ang II which may contribute to CNV development in hypertensive dry AMD patients.

Our results indicated that hypertension-induced Ang II increases MCP-1 and MCP-1P expression in mouse RPE-choroid through AT1 receptor. *In vitro*, MCP-1 and MCP-1P expression was up-regulated by Ang II in RPE cells. Moreover, MCP-1 induced expression of MCP-1P in RPE cells, which led to cEC tube formation (Figures 5-7) (Marin-Castano *et al.*^[140] IOVS 2013; ARVO E-Abstract 6089). Therefore, our data support the hypothesis that Ang II, through MCP-1/MCP-1P may contribute to CNV, proposing a possible mechanism linking hypertension and CNV, which can provide new targets for more effective early preventive and novel therapeutic interventions.

DR AND THE RAS

The incidence of DR is alarming. A recent study emphasizes that 93 million people have DR, and that about 17 million have the blinding form of the disease^[141]. Patients with type 1 or type 2 diabetes are at risk for the development of DR. The longer a person has diabetes, the more likely they are to develop DR^[142]. DR is classified into two types: (1) non-proliferative DR (NPDR), the early state of the disease. In NPDR, the blood vessels in the retina are weakened causing tiny bulges called microaneurysms. The microaneurysms may leak fluid into the retina, which may lead to swelling of the macula; and (2) proliferative DR (PDR), which is the more advanced form of the disease. At this stage, the retina becomes oxygen deprived. New blood vessels can start to grow in the retina and into the vitreous causing clouding vision. If left untreated, PDR can cause severe vision loss and even blindness^[143]. The progression to PDR looks like to be a result of tissue ischemia and the consequent increase in the production of angiogenic growth factors such as VEGF.

The report that some components of the RAS are augmented in blood and eyes from DR patients^[46,144,145], suggests the RAS may be implicated in the pathogenesis

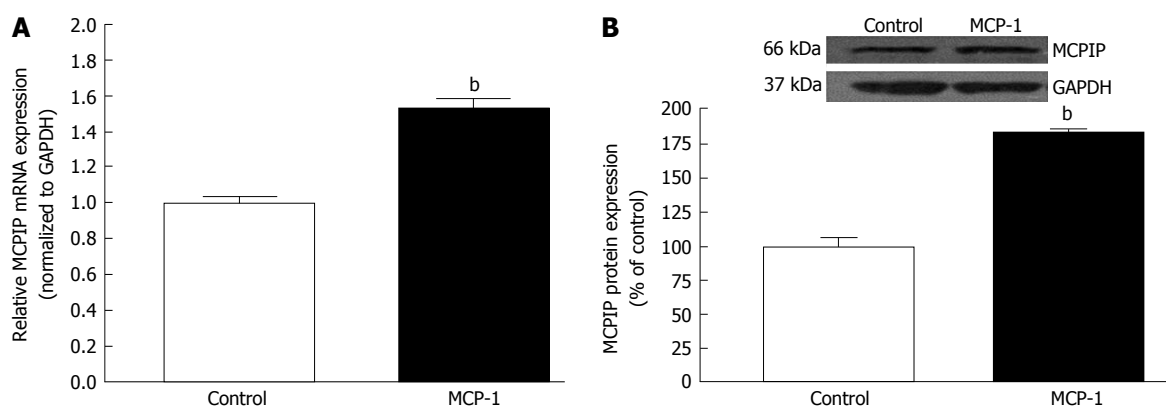


Figure 6 Monocyte chemoattractant protein up-regulates monocyte chemoattractant protein-1 induced protein expression in ARPE-19 cells^[140]. Monocyte chemoattractant protein-1 (MCP-1) increases MCP-1 induced protein (MCPIP) mRNA (A) and MCPIP protein expression (B) in human retinal pigment epithelium cells. The maintenance medium was deprived of phenol red for 2 d. Medium FBS content was then brought down from 10% to 1% for 1 d. Subsequently, cells were treated with 50 pg/mL MCP-1 for 24 h in a medium supplemented with 0.1% FBS. Cell homogenates were collected to assess MCPIP expression by real-time PCR and Western blot. GAPDH was used as control. Data are mean \pm SE ($n = 4$). ^b $P < 0.01$ vs control cells; FBS: Medium with fetal bovine serum; PCR: Polymerase chain reaction; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

of DR^[28]. An increase of angiotensinogen, ACE, ACE2, and AT1R in retinas from diabetic animals was described previously^[35,146,147]. Up to now, research addressed to find a link between the RAS and retinopathy has been based on the retinal microvasculature. Strong evidence supporting a role of Ang II in pericytes and endothelial cells in the retinal microvasculature has been shown. Ang II has a mitogenic effect on retinal endothelial cells^[23,59,148]. This peptide also decreases the expression of pigment epithelium derived growth factor^[148] and enhances proliferation of endothelial cells in retina through VEGF up-regulation^[23,149]. Moreover, glucose ingestion by the retinal tissue might be instantly regulated by Ang II^[150,151]. This increase in glucose in turn could induce VEGF expression and potentiates the effect of Ang II on VEGF expression as demonstrated previously in vascular smooth muscle cells^[152]. Since it is clear that reactive oxygen species (ROS) contribute to cellular damage in DR by inducing VEGF^[153,154], and that both Ang II and high glucose can lead to ROS formation,^[155,156] ROS may be a common pathway linking a synergistic effect between Ang II and high glucose on the activation of VEGF.

The actions of Ang II on the retinal vasculature have been well described in pericytes. These microvascular cells are incriminated in the regulation of capillary tone^[157], and it has been suggested they have other extra roles such as preservation of microvascular homeostasis^[149]. For instance, death of pericytes has been linked to the initial sign of DR. It has been reported that Ang II uncouples pericytes from the vasculature^[48,158]. Studies *in vitro* have shown activation of pericyte migration by Ang II through the AT1R^[159,160]. Moreover, Ang II also has an effect on pericyte viability, by increasing apoptosis^[33,59]. Therefore, it is evident that Ang II impacts the retinal microvasculature. Research in diabetic animals showed a reduction in the retinal microvascular injury by exposure to ACE inhibitors and AT1R blockers. These data revealed a decrease in the vascular leakage, acellular capillaries formation, VEGF production^[161-164], leukostasis and adhesion

molecules^[164-167]. Comparable advantages were observed in different animal models of diabetes, which were treated with renin inhibitors^[167], PRR inhibitor^[35], and gene delivery of ACE2^[160] respectively. Diabetes may also affect neuronal retina in DR. For example, diabetic retina may reveal releasing of pro-inflammatory factors by microglia^[168], death of retinal neurons^[169], apoptosis of ganglion cells^[170], glial dysfunction^[170] and photoreceptors loss^[171]. These pathological neuronal effects may be translated to electrophysiological abnormalities^[172-174]. Color vision, contrast sensitivity and dark adaption^[24,175] can be altered by diabetes before the presence of any apparent pathological sign in the vessels^[175]. Given that treatment with ACE inhibitors and AT1R blockers decreases these deficits in retinal function^[176-179], the advantages of RAS blockade could extend to non-vascular cells.

It is also interesting to note that discovery of other important players on the RAS such as ACE2 and Ang (1-7) has resulted in the emerging new role ascribed to these RAS components beyond the classic ACE/Ang II/AT1R axis of the RAS^[179-180]. Nevertheless, the force of this novel axis stays inadequately elucidated^[180,182-184]. This new protective axis antagonizes the classic role of the vasoconstrictor axis. Thus, it was assumed that a disproportion in the vasoprotective/vasodeleterious axis of the RAS, could result in the development and progression of DR. Many studies in non-ocular tissues have emphasized the beneficial effect of the balance displacement of the RAS towards the ACE2/Ang (1-7)^[180,185-189]. Therefore, activation of the vasoprotective axis is currently considered to be part of the beneficial actions of ACEi and ATRs blocker drugs^[180,182], which neutralize the actions of Ang II, in spite of its origins of generation^[146].

High blood pressure is a great risk factor for DR. Several studies have been addressed to elucidate if the contribution of the Ang II to the development of DR is *via* blood pressure dependent or independent. This is an intricate search, given that blockers of some compound of the RAS decrease both blood pressure and the actions

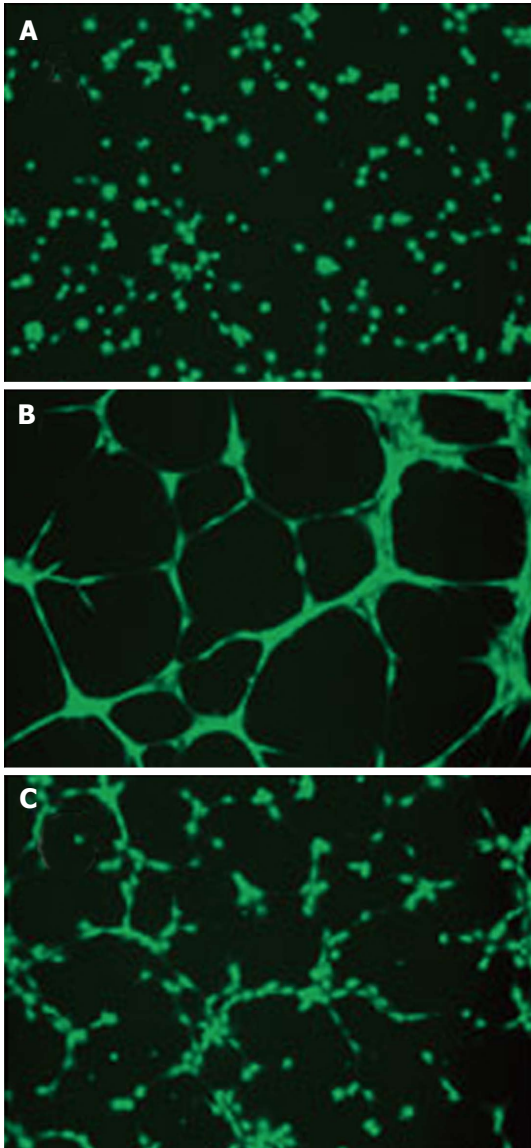


Figure 7 Conditioned medium collected from human ARPE-19 cells exposed to Ang II promotes tube formation in choroidal microvascular endothelial through AT1 activation^[140]. Cells were exposed to: (1) Ang II alone; or (2) Ang II in combination with candesartan for 24 h, supernatants were collected after treatment and human choroidal microvascular endothelial (cECs) were treated with the supernatants for 24 h. Thereafter, cells were trypsinized and then seeded (42000 cells/cm²) on a 24-well polystyrene plate coated with Geltrex™ (50 µL/cm²) according to the manufacturer's protocol followed by incubation in EBM medium for 24 h at 37 °C in 5% CO₂. At 16 h post-seeding, 2 µg/mL of Calcein, AM (Invitrogen, Cat # C3099), was added directly to the culture well and allowed to incubate for 20 min (37 °C, 5% CO₂). Cells were visualized using a fluorescence microscope. A: Control; B: cECs exposed to conditioned medium from Ang II-treated ARPE-19 cells; C: cECs treated with medium collected from treated retinal pigment epithelium cells. EBM: Endothelial cell basal; AM: Acetoxymethyl.

of the Ang II at cellular levels. Studies in Ren-2 rat with hypertension showed that both AT1R and β -adrenergic blockade regularize blood pressure^[158]. Nevertheless, the retinal vascular pathology only becomes better using AT1R blockers. Additional determination of the blood pressure-independent effects of the RAS blockade in DR is crucial for diabetic patients without hypertension.

The mechanism(s) by which the RAS exerts its effects in the retina are being investigated. There is proof that hypertension and mechanical stretch up-regulate the RAS and VEGF expression^[190]. It has been previously demonstrated an increase of VEGF in the RPE^[191] and in retinal endothelial cells^[192] due to mechanical stretch. Moreover, rats with hypertension showed increased expression of the VEGFR-2 in the retina^[191]. Therefore, it could be probable that the decrease in VEGF reported in DR^[193] following RAS blockade could be due to the antihypertensive properties of this treatment, rather than, suppression of the growth factor effects of Ang II. Moreover, given that a relationship between ROS and cellular damage in DR has been demonstrated and the fact that ROS production is induced by Ang II^[153,154,194,195], it is likely that ROS are essential in the pathogenesis of DR. The main origin of the ROS is nicotinamide adenine dinucleotide phosphate (NADPH, or NOX) and ROS originated from NOX have been associated with the development of DR^[196,197]. Ang II modulates NOX to generate ROS^[194,198]. However, the connection between the RAS and NOX in retinopathy is not completely clarified yet^[199,200]. Obviously, the link involving RAS and NOX in DR guarantees further study.

Clinical trials evaluated the influence of Ang II in the development and progression of DR. To elucidate this, three major studies addressed to evaluate the blockade of the RAS were done: (1) the DIabetic RETinopathy Candesartan trial^[201-204]; (2) the Appropriate Blood Pressure Control in Diabetes trial^[205]; and (3) the Action in Diabetes and Vascular Disease Controlled Evaluation (ADVANCE) trial^[206]. The first study showed that candesartan, an AT1R blocker, modestly avoid the evolution of retinopathy in type 1 diabetic patients without hypertension. From another point of view, this AT1R blocker caused reversion of retinopathy in type 2 diabetic patients in a 34% regression of retinopathy and decreased the risk of microaneurysm evolution in both types of diabetes^[202]. The second trial study, showed notably benefit for RAS blockade^[203], whereas the ADVANCE study reported that treatment with a combination of an ACE inhibitor and a diuretic, did not affect the retinopathy risk^[205]. I summary, these data document the influence of Ang II in the development of DR. Further evaluation of the RAS blockade in DR is still to be determined.

CONCLUSION

Hypertension is a potential link between cardiovascular pathologies and eye diseases. A large amount of information has demonstrated the presence of a RAS in the retina which is greatly spread in the vasculature. To date, findings from epidemiological studies indicate an association between AMD and hypertension. Moreover, studies *in vitro* and *in vivo* show that Ang II contributes to sub-RPE deposit formation and CNV development and that these events can be improved by Ang II receptor blockers (ARBs). However, the utility of ARBs for the treat-

ment of eye AMD is still to be determined. In terms of DR, there is documented evidence showing a clear contribution of Ang II to the development of this disease. Therefore, the use of ARBs can confer retinoprotection and arrest the progression of DR.

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Is ABO blood group truly a risk factor for thrombosis and adverse outcomes?

Shan Zhou, Ian Welsby

Shan Zhou, Ian Welsby, Department of Anesthesiology, Duke University Medical Center 3094, Durham, NC 27710, United States

Shan Zhou, Department of Anesthesiology, Fuwai Cardiovascular Hospital, Chinese Academy of Medical Science, Peking Union Medical School, Beijing 100037, China

Author contributions: Zhou S and Welsby I contributed to this paper.

Correspondence to: Dr. Ian Welsby, Department of Anesthesiology, Duke University Medical Center 3094, 2301 Erwin Road, Durham, NC 27710, United States. ian.welsby@dm.duke.edu

Telephone: +1-91-96682699 Fax: +1-91-92872720

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Abstract

ABO blood type is one of the most readily available laboratory tests, and serves as a vital determinant in blood transfusion and organ transplantation. The ABO antigens are expressed not only on red blood cell membranes, determining the compatibility of transfusion, but also on the surface of other human cells, including epithelium, platelet and vascular endothelium, therefore extending the research into other involvements of cardiovascular disease and postoperative outcomes. ABO blood group has been recognized as a risk factor of venous thrombosis embolism since the 1960's, effects now understood to be related to ABO dependent variations are procoagulant factor VIII (FVIII) and von Willebrand factor (vWF) levels. Levels of vWF, mostly genetically determined, are strongly associated with venous thromboembolism (VTE). It mediates platelet adhesion aggregation and stabilizes FVIII in plasma. Moreover, many studies have tried to identify the relationship between ABO blood types and ischemic heart disease. Unlike the clear and convincing associations between VTE and ABO blood type, the link between ABO blood type and ischemic heart disease is less

consistent and may be confusing. Other than genetic factors, ischemic heart disease is strongly related to diet, race, lipid metabolism and economic status. In this review, we'll summarize the data relating race and genetics, including ABO blood type, to VTE, ischemic heart disease and postoperative bleeding after cardiac surgery.

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Key words: ABO blood group; Venous thrombosis; Ischemia disease; Cardiac surgery; Outcomes

Core tip: In this review, we updated the reports regarding the associations between ABO blood groups and venous thrombosis, ischemic heart disease as well as postoperative outcomes after cardiac surgery. ABO blood group is clearly associated with venous thromboembolism whereas critical review of the literature reveals a more controversial relationship with atherosclerosis, arterial thrombosis and postoperative outcomes.

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INTRODUCTION

The ABO group of human red cell antigens was discovered by Karl Landsteiner in 1900. ABO antigens are carbohydrate molecules that are the major determinants of the compatibility of red cell transfusions. Naturally occurring, complement fixing IgM antibodies are formed against the A and B antigens in individuals that do not express them on their red cell surfaces and therefore recognize them as foreign antigens. Each individual inherits

Table 1 The incidence of ABO phenotypes in populations from different racial backgrounds

Race	Blood group phenotype O ¹ (O ² rare)	Blood group genotypes				
		A ¹	A ²	B	A ¹ B	A ² B
Caucasian	44%	33%	10%	9%	3%	1%
Asian	43%	27%	Rare	25%	5%	Rare
African	49%	19%	8%	20%	3%	1%

Illustrations: Sub-group A2 expresses less A antigen on the red cell surface and has been referred to as “weak” A.

Table 2 The association of ABO genotype with von Willebrand factor and factor VIII levels is presented with categorization by von Willebrand factor levels

	Genotype	Median value	
		vWF	FVIII
Low	O ¹ O ¹	69%	75%
Medium	A ¹ O, A ² O, BO	89%	96%
High	AA, BB, A ¹ B	120%	117%
Highest	A ² B	169%	112%

vWF: Von Willebrand factor; FVIII: Factor VIII.

two ABO alleles. The A and B alleles encode separate glycosyltransferase that add N-acetylgalactosamine and D-galactose of the “H” antigen (group O determinant), converting it into A and B antigens respectively. However, as the O allele does not express either A or B transferase enzymes, continued expression of the unaltered H antigen is the phenotypic marker of the O blood group^[1]. The ABO antigens are expressed not only on red blood cell membranes, determining the compatibility of transfusion, but also on the surface of other human cells, including epithelium, platelet and vascular endothelium^[2], therefore extending potential pathophysiology into other areas of cardiovascular disease and postoperative outcomes.

Expression of the different ABO phenotypes is partially dependent on racial origin as shown in Table 1, with Group O generally being the most common blood group^[3]. Blood groups are basically described by phenotypes, because historically blood groups are determined by commercial antibodies that recognize A and B antigens. By this detection method, both AO and AA genotypes (A¹O¹, A¹A²) will be identified as group A, while BO and BB genotypes as group B. In this review, we updated the reports regarding the associations between ABO blood groups and venous thrombosis, ischemic heart disease as well as postoperative outcomes in terms of both ABO phenotype and genotype.

ABO AND VON WILLEBRAND FACTOR

Von Willebrand factor (vWF) has two major biological forms and the high molecular weight vWF (HMW vWF) is hemostatically more active than the low molecular weight vWF (LMW vWF)^[4]. HMW vWF mediates the interaction between platelets and damaged areas of the blood vessel wall, while LMW vWF acts as a specific car-

rier molecule for procoagulant factor VIII (FVIII), thereby localizing FVIII to the site of any vascular injury. Both are essential for normal hemostasis^[5,6].

Plasma vWF levels are generally reported to be approximately 25% higher in non-O blood individuals^[7]. Synthesized in endothelial cells and megakaryocytes, the HMW vWF, enters the plasma from platelet granules following platelet activation and degranulation at the site of tissue injury, or alternatively being stored in endothelial cell Weibel-Palade bodies, then secreted in response to thrombin, fibrin or histamine stimulation^[8]. vWF molecule has three binding sites, platelet glycoprotein 1b binds to A1 domain, while collagen binds to A3 domain, forming the primary hemostatic clot^[9,10]. The A2 domain binds to ADAMTS13 and is responsible for vWF cleavage (Figure 1).

Clinical observations that the severity of bleeding in mild von Willebrand's disease was exaggerated for group O patients led to the recognition of an ABO dependent variation in vWF levels^[11-13]. A formal linkage analysis showed the effect of ABO blood type on von Willebrand factor is a direct functional effect of the ABO locus, rather than linkage disequilibrium between the ABO locus and another unidentified VWF regulation locus^[14]. vWF levels can also influence procoagulant FVIII levels since vWF is a carrier molecule that protects FVIII from proteolysis in plasma.

Moeller *et al.*^[15] compared vWF and FVIII levels in individuals of different ABO phenotype and found ascending order O < A < B < AB for vWF level and O < A < AB < B for FVIII level. This effect becomes more nuanced when considering the specific genotypes that result in ABO phenotypes, as illustrated in Table 2. Within A and B phenotypes, vWF concentrations in AA or BB are slightly higher than AO or BO^[16] and A¹ and B alleles are found to be associated with higher vWF and FVIII levels, while A² is comparable to O allele^[6,11,13,17].

MECHANISM FOR ABO RELATED VARIABILITY IN VWF LEVELS

There is no direct evidence demonstrating that the ABO locus is associated with vWF synthesis^[8], therefore efforts to elucidate the association between ABO and vWF have focused on vWF metabolism and cleavage. ADAMTS13 cleavages HMW vWF to LMW vWF^[8,15,18,19], thereby modulating the tendency of vWF to cause platelet aggregation and thrombus formulation^[20]. The biological importance of this is exemplified by thrombotic thrombocytopenic purpura (TTP). In TTP, autoantibodies neutralize ADAMTS13 leading to diffuse microvascular thrombosis from the unregulated action of HMW vWF. This extreme example leads to a proposed mechanism for the ABO group related modulation of vWF levels and therefore tendency to thrombosis. While A, B and H antigens are more commonly known to be expressed on the cell surfaces of erythrocytes and various exocrine cells, they are also expressed on the vWF molecule. The

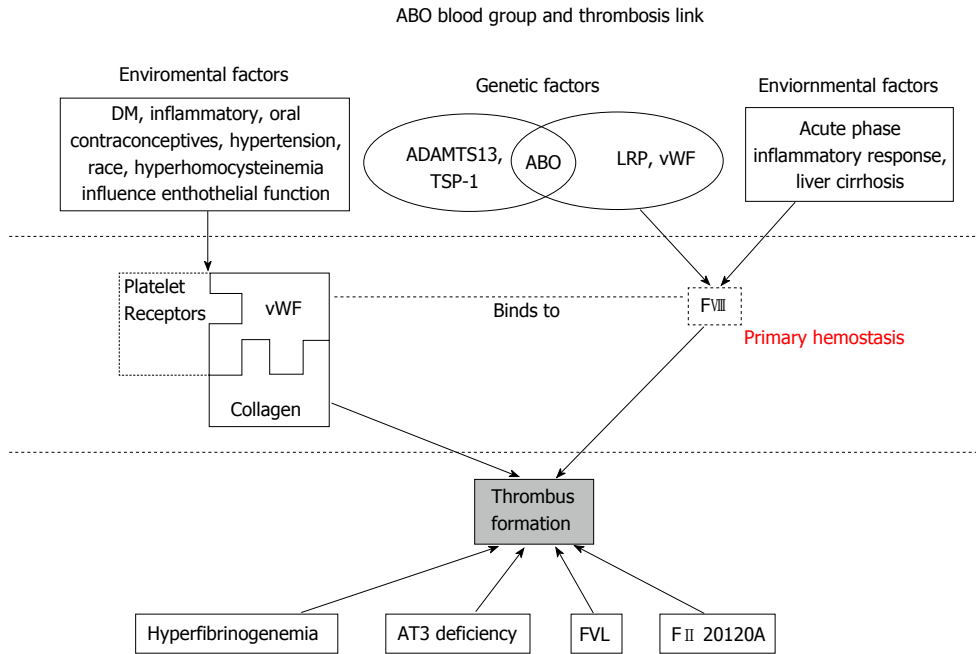


Figure 1 Genetic and environmental factors that contribute to increased levels of von Willebrand factor and factor VIII and risk of thrombus formation. AD-AMTS13: A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; AT3: Antithrombin III; DM: Diabetes mellitus; F II: Prothrombin gene mutation 20120A; FVIII: Factor VIII; FVL: Factor V leiden; LRP: Lipoprotein receptor-related protein; TSP-1: Thrombospondin-1; vWF: Von Willebrand factor.

location of the A, B and H antigens on the vWF molecule is thought to be close to the A2 domain binding site for ADAMTS13 and that A and B antigens reduce ADAMTS13 binding and, therefore, cleavage^[8,21]. Some studies confirmed this hypothesis by providing evidence that the proteolytic effect of ADAMTS13 on vWF was significantly faster in O group (only H antigen expression) than in non-O groups with A and B antigen expression^[22,23]. Factors other than ABO group can also modify vWF metabolism which may limit the direct association of ABO group with vWF levels and thrombosis, explaining some inconsistencies in the various studies we report. For example, thrombospondin-1 (TSP-1) has been reported to control vWF multimer size by both directly cleavage and indirectly, competing with ADAMTS13^[24,25]. Thus, any genetic factors influence cleavage (ABO blood type, ADAMTS13 and TSP-1) and environmental risk factors that affect endothelial cell function, such as age, diabetes mellitus, hypertension, inflammatory and oral contraceptive drugs, all contribute to the complex risk factors leading to clinical thrombosis. This concept is illustrated in Figure 1.

The link between ABO blood group, H antigen expression and lower vWF levels has been well established above. How this translates into a clinically relevant risk of thromboembolism manifesting either as venous thromboembolism or coronary artery thrombosis is discussed in detail below.

VENOUS THROMBOEMBOLISM

Venous thromboembolism (VTE) includes deep vein thrombosis and pulmonary embolism and is a serious

medical condition with a historical mortality rate of 10% and 15% respectively^[26]. ABO blood group has been recognized as a risk factor since the 1960's, effects now understood to be related to ABO dependent variations are procoagulant FVIII and vWF levels. Levels of vWF, mostly genetically determined, are strongly associated with VTE. It mediates platelet adhesion aggregation and stabilizes F VIII in plasma. In a healthy state, twin studies showed 75% of variance in plasma vWF levels result from genetic determinants^[27], 30% of which are associated with ABO blood type^[28]. Other non-genetic factors, such as aging, diabetes, free radical formation and inflammation, may have a more important role during acute illnesses or during the perioperative period^[29]. As shown in Figure 1, environmental causes of endothelial dysfunction can greatly affect vWF levels.

Numerous studies have reported that individuals with non-O blood types had a higher risk of VTE compared to their O counterparts^[30-34]. According to Wiggins, compared to O¹O¹ group, AB diplotype category has the highest VTE rate, followed by B allele and A¹ allele^[13]. Other rare genotypes like A³, A^x, A^a, B³, B^a were less amenable to statistically meaningful comparison in this study. These observations were supported by genotype association studies that showed H-antigen rich genotypes (O¹O¹, O¹O², O¹A²) have a lower incidence of VTE than H-antigen poor genotypes (A¹B, O¹A¹, O¹B)^[17,35,36], establishing ABO blood type as an important risk factor for VTE^[37].

In Figure 1, various genetic and environmental factors affecting vWF levels are presented. What's more, FVIII, circulating bound to vWF, also plays a crucial and independent role in the propagation phase of coagulation

Table 3 Outline of the main studies describing the association of ABO blood type and manifestations of atherosclerotic heart disease

Ref.	Population	Sample size	Outcome(s)	Findings
Garrison <i>et al</i> ^[44]	United States		"Cardiovascular disease"	O showed the lowest incidence
Whincup <i>et al</i> ^[45]	United Kingdom (men only)	7662	CAD	Individuals with A blood type has higher incidence of CAD (RR = 1.21, CI: 1.01-1.46)
Rosenberg <i>et al</i> ^[46]	United States (young women)	225 MI vs 802 controls	MI	Blood group A was associated with MI
Lee <i>et al</i> ^[47]	Taiwan (young patients)	136 CAD vs 129 without CAD	CAD and MI	Group A was associated with increased risk of CAD (OR = 2.61, CI: 1.11-6.14) and MI (OR = 3.53, CI: 1.21-10.29)
Sari <i>et al</i> ^[48]	Turkish	476 MI vs 203 healthy control	MI	ABO blood type is not associated with development of MI
Carpeggiani <i>et al</i> ^[49]	Italy	4901	MI and CAD	Group non-O is associated with increased mortality in patients with CAD, groups A and B prevail in MI
Nydegger <i>et al</i> ^[50]		177 patients vs 89 control	MI	B allele carriers had higher MI (OR = 2.7, CI: 1.1-6.8)
Stakisaitis <i>et al</i> ^[51]	Lithuania	441	CAD	B blood group can be related with CAD in women
Meade <i>et al</i> ^[52]	United Kingdom	1393 men with 178 IHDs	CAD and MI	Incidence was significantly higher in blood group AB
Mitchell <i>et al</i> ^[53]	United Kingdom		"Cardiovascular disease"	Towns with higher prevalence of group O have higher rate of cardiovascular mortality
Biswas <i>et al</i> ^[54]	India	250 CAD vs 250 controls	CAD	Group O increases the risk of CAD
Amirzadegan <i>et al</i> ^[55]	Iran	2016 patients	CAD	No correlation
Biancari <i>et al</i> ^[56]	Finland	1152 CABG patients	MI	No correlation
He <i>et al</i> ^[57]	United States	89501	Coronary heart disease	AB group has highest CAD risk, followed by groups B, A and O

CAD: Coronary artery disease; MI: Myocardial infarction.

activation^[6]. Since vWF is the plasma co-carrier of FVIII, ABO blood type, by altering vWF levels, also exerts an effect on FVIII levels. Tirado *et al*^[34] demonstrated that genetic factors explain 40% of the variance of FVIII levels; other studies further identified a quantitative trait locus and the ABO locus as two major genetic factors underlining the variability of FVIII levels^[14,38]. Unconnected to ABO group, lipoprotein receptor-related protein has also been identified to be associated with degradation of FVIII, another consideration when evaluating variance in FVIII levels^[39]. In summary, while ABO blood type and vWF levels are two important factors commonly known to modulate FVIII plasma level, the biology determining FVIII is a complex interaction of genetic and environmental factors as illustrated in Figure 1.

However, FVIII may have some effect independent of vWF. Some studies demonstrated that a high FVIII level is persistent beyond the acute phase state^[40,41], representing a potential risk factor for delayed or recurrent thrombosis. In addition, Morange *et al*^[17] described a residual statistical effect of ABO blood group on FVIII levels after adjustment for vWF levels, postulating that FVIII is an independent VTE risk factor^[29,34]. Additionally, FVIII was reported to be associated with recurrent disease^[34], consistent with reports that non-O carriers had a higher incidence of VTE recurrence than O carriers^[42,43].

ISCHEMIC HEART DISEASE

Unlike the clear and convincing associations between VTE and ABO blood type, the link between ABO blood type and ischemic heart disease is less consistent and may be confusing. In part this can be due to the inclusion of different end-points that may represent different disease

processes, such as angina/atherosclerosis (less likely ABO/vWF related) or myocardial infarction (MI)/coronary thrombosis (more likely ABO/vWF related). The pathogenesis of coronary artery disease (CAD) involves the progression of an atherosclerotic disease process, whereas MI (or acute coronary syndrome) results from a platelet rich thrombus forming on abnormal endothelium diseased by the atherosclerotic process. Platelet rich thrombi (MI) are reliant on primary hemostasis, whereas the mechanism linking ABO group to CAD is less obvious. However, it is important to evaluate ABO group as a risk factor for both these devastating conditions: CAD and MI.

Many studies show that non-O group have higher incidence of ischemic heart disease (Table 3). The Framingham Heart study, and others, suggested A blood type has increased risk of CAD^[44-46] and MI^[47]; more specifically, A blood group seems to be related to early CAD detection^[47,48] and predominates in patients with MI^[49]. Other studies noted groups B^[50,51] or AB^[52] have higher incidence of CAD. Conversely, Mitchell^[53] reported that towns with a higher prevalence of group O have higher rates of cardiovascular mortality and an Indian study with moderate sample size also showed O blood group is more frequent in CAD and increased the risk of CAD^[54]. Further studies do not identify any association between blood type and CAD^[55,56]. Based on these inconsistent results and relative small sample sizes. He^[57] conducted a meta-analysis of two large, prospective studies consisting of 89501 participants, and found the highest risk of CAD was observed in blood group AB, followed by group B, A and O. This is consistent with what we know about ABO related vWF/FVIII levels with the highest in group AB, followed by group B, A and O. According to

this meta-analysis, non-O group has an 11% increased risk of CAD, an association not altered by adjusting for other co-morbidities. There was, however, no difference in survival and, paradoxically, a trend towards increased mortality and/or non-fatal myocardial infarction in O blood type patients.

The relationship between ABO genotype and CAD has also been investigated. Wiggins *et al.*^[13] reported an 18% increased MI risk associated with A¹¹ allele carriers compared to O¹O¹ homozygotes, but no other associations were found between B or AB alleles and MI, possibly due to underpowering as B and AB groups are relatively rare. An investigation of postmenopausal women suggested A or B allele carriers almost had two-fold incidence of acute ischemic heart disease compared to OO^[58]. Similarly, Nydegger *et al.*^[50] showed a three-fold risk of MI with the presence of B allele (genotype AB, BB or BO) compared to non B allele (genotype OO, AO, AA) in a smaller case-control study. Another study^[59] with angiography showed O¹ allele carriers had a 39% decreased risk of MI compared to non O¹. More obviously, von Beckerath *et al.*^[59] found a dose-dependent effect with carriage of one or two O¹ alleles being associated with decreased risks of acute MI. However, a recently published study by Reilly *et al.*^[60] argued that ABO locus did not predict MI in patients with known CAD, but was strongly associated with the presence of CAD in two large genome wide association studies. Whether ABO alleles are associated with the development of MI or only the presence of CAD is not yet clearly defined. It is much easier to investigate the risk factors for CAD prevalence in a cross-sectional study than to evaluate the incidence of MI with a prospective design, as the latter requires a stable cohort with years of detailed follow-up. Currently, the association of MI and ABO blood group has only been well reported in survivors of MI events. This introduces bias, as patients may suffer an asymptomatic MI, not present at hospital, or die before diagnosis.

There are some mechanisms proposed to explain the association between ABO blood type and CAD, but a unifying theory remains elusive. Along with fibrinogen, vWF may play a role in the progression of atherosclerosis by promoting platelet aggregation and adhesion^[21]. On the other hand, blood group A has been noted to have higher levels of cholesterol and low density lipoprotein^[61], which may partly explain the association with an increased risk of CAD. Additionally, the ABO locus was recently reported to be associated with CAD related inflammatory makers, including intercellular adhesion molecule-1, soluble P-selectin^[62], soluble E selectin^[63] and tumor necrosis factor- α ^[53]. Still, the interactions among genetic factors (known genes increasing susceptibility to CAD and the ABO locus) and environmental factors conferring risk for CAD and MI are complicated. It is unclear which ABO phenotypes or genotypes increase CAD and/or MI risk; this risk may differ for the incidence of CAD or MI and survival following MI.

CARDIAC SURGERY

Our group performed a retrospective study to evaluate the relationship between ABO blood types and postoperative bleeding in cardiac surgical patients. This was based on the hypothesis that lower circulating vWF levels seen with group O may reduce primary hemostasis resulting in increased postoperative bleeding. While group O did have impaired baseline measures of primary hemostasis and required less heparin and protamine for perioperative anticoagulation, the result showed no difference of postoperative bleeding between different blood groups^[20]. Limitations of such perioperative studies are the lack of intermediate, mechanistic measures of factor levels and the confounding effects of the acute phase response that may drown out an ABO effect. Also, the classification by phenotype is limited. For example, the A²O genotype with low vWF levels and the A¹A¹ genotype with high vWF levels are both classified as group A. In addition, the statistically convenient categorization into O and non-O phenotype is flawed for the same reason, blurring comparison between H antigen rich and H antigen poor genotypes that have been shown to drive the association between ABO blood type and outcome. As an alternative approach, we have preliminary results suggesting that the AB phenotype (no H antigen) requires less perioperative transfusion than non-AB phenotypes and this is associated with better postoperative survival for the rare AB group. These findings require confirmation with prospective study.

CONCLUSION

In summary, ABO blood group is an important determinant of vWF and FVIII levels which in turn confer a clear risk of increased VTE with the higher levels seen in the non-O blood types. The associations are far less clear for CAD and MI but a similar pattern emerges with most studies finding group O to be at lower risk. In terms of perioperative bleeding and transfusion, a possible reciprocal for thrombosis, further work needs to be done to determine a consistent ABO effect.

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Cardiac manifestations in systemic sclerosis

Sevdalina Lambova

Sevdalina Lambova, Department of Propedeutics in Internal Medicine, Medical University, Plovdiv 4002, Bulgaria

Author contributions: Lambova S performed the review and analysis, and wrote the paper.

Correspondence to: Sevdalina Lambova, MD, PhD, Department of Propedeutics in Internal Medicine, Medical University, 15A Vasil Aprilov Blvd, Plovdiv 4002,

Bulgaria. sevdalina_n@abv.bg

Telephone: +359-602411 Fax: +359-602273

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Abstract

Primary cardiac involvement, which develops as a direct consequence of systemic sclerosis (SSc), may manifest as myocardial damage, fibrosis of the conduction system, pericardial and, less frequently, as valvular disease. In addition, cardiac complications in SSc may develop as a secondary phenomenon due to pulmonary arterial hypertension and kidney pathology. The prevalence of primary cardiac involvement in SSc is variable and difficult to determine because of the diversity of cardiac manifestations, the presence of subclinical periods, the type of diagnostic tools applied, and the diversity of patient populations. When clinically manifested, cardiac involvement is thought to be an important prognostic factor. Profound microvascular disease is a pathognomonic feature of SSc, as both vasospasm and structural alterations are present. Such alterations are thought to predict macrovascular atherosclerosis over time. There are contradictory reports regarding the prevalence of atherosclerosis in SSc. According to some authors, the prevalence of atherosclerosis of the large epicardial coronary arteries is similar to that of the general population, in contrast with other rheumatic diseases such as rheumatoid arthritis and systemic lupus erythematosus. However, the level of inflammation in SSc is inferior. Thus, the atherosclerotic process may not be as aggressive and not easily detectable in smaller studies. Echocardiography (especially tissue

Doppler imaging), single-photon emission computed tomography, magnetic resonance imaging and cardiac computed tomography are sensitive techniques for earlier detection of both structural and functional scleroderma-related cardiac pathologies. Screening for subclinical cardiac involvement *via* modern, sensitive tools provides an opportunity for early diagnosis and treatment, which is of crucial importance for a positive outcome.

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Key words: Systemic sclerosis; Cardiac involvement

Core tip: The prevalence of primary cardiac involvement in systemic sclerosis (SSc) is difficult to determine, as it can manifest as myocardial damage, fibrosis of the conduction system, pericardial and valvular disease. When clinically manifested, cardiac involvement is thought to be an important prognostic factor. Echocardiography, magnetic resonance imaging and computed tomography are sensitive techniques for earlier detection of structural and functional SSc-related cardiac pathologies. Screening for subclinical cardiac involvement provides an opportunity for early diagnosis and treatment, which is of crucial importance for a positive outcome.

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INTRODUCTION

Systemic sclerosis (SSc) is a chronic multisystem disease characterized by microangiopathy, fibrosis of the skin and internal organs, and autoimmune disturbances. Two major subsets are recognized, namely, SSc with limited cutaneous involvement (skin thickening is localized to the face, neck and extremities distal to elbows and knees) and

SSc with diffuse cutaneous involvement (skin thickening also involves the extremities proximal to elbows and knees, chest, abdomen and back).

Primary cardiac involvement, which develops as a direct consequence of SSc, may manifest as myocardial involvement, fibrosis of the conduction system, pericardial and, less frequently, as valvular disease. Furthermore, cardiac complications in SSc may develop as a secondary phenomenon due to pulmonary arterial hypertension (PAH), interstitial lung disease, and kidney pathology^[1,2].

PRIMARY CARDIAC INVOLVEMENT IN SSc

The prevalence of the primary cardiac involvement in SSc is difficult to determine due to the numerous possible cardiac manifestations, the applied diagnostic tools and diverse patient populations. Of note, the results of histologic studies, which frequently reveal the presence of myocardial involvement, often disagree with those of the clinical studies, performed with different assessment techniques^[3].

Clinical examination and routine non-invasive investigations, such as electrocardiogram and thoracic X-ray, are applied in the everyday cardiac assessment, but their sensitivity is low^[1,4,5]. Echocardiography [especially tissue Doppler imaging (TDI)], cardiac computed tomography (CT), single-photon emission CT (SPECT), magnetic resonance imaging (MRI), positron emission tomography (PET), and radionuclide ventriculography are sensitive techniques for earlier detection of both structural and functional scleroderma-related cardiac pathologies^[1]. In the recent years, with the improvement of the prognosis of scleroderma renal crisis (SRC), pulmonary and cardiac involvement are the main causes for disease-associated mortality in SSc. Signs for cardiac involvement have been detected with a prevalence of 15% in a cohort of 953 patients with diffuse cutaneous SSc based on clinical findings, echocardiography, electrocardiography, or Holter monitoring^[6]. Asymptomatic decreases in the ejection fraction, asymptomatic pericardial effusion, or asymptomatic arrhythmias were not considered as significant heart manifestations in this study. Disease-associated mortality was found to be 20% in a ten-year follow-up^[5,6]. The greatest impact occurred in the first five years (14% mortality rate).

Conventional echocardiography is used for cardiac assessment in most studies. Depressed left ventricle (LV) contractility has been reported in only a few patients, whereas up to 40% present with relaxation abnormalities, valvular regurgitation and possible right ventricular (RV) pathology^[3]. A recent analysis of organ involvement in a large cohort of 9165 SSc patients from the European League Against Rheumatism Scleroderma Trials and Research database revealed that diastolic dysfunction was among the most frequent features (17.4%). Palpitations were also a common finding in 23.7% of the cases, whereas conduction blocks were detected in 11% by

electrocardiography^[7]. In a cohort of 1012 Italian SSc patients, the prevalence of cardiac symptoms of arrhythmia was 35%^[3].

LV systolic dysfunction is among the rarest findings in SSc patients. In a large multi-centered study, which included 570 SSc patients, the prevalence of LV systolic dysfunction was found to be 1.4%, whereas LV hypertrophy and LV diastolic dysfunction were observed in 22.6% and 17.7% of patients, respectively^[8]. In a recent, large European League Against Rheumatism Scleroderma Trials and Research study (including 7073 consecutive SSc patients with a mean age of 56 ± 14 years) the prevalence of reduced LV ejection fraction was found to be 5.4%^[9].

Of note, cardiac MRI detected heart pathologies in up to 75% (39/52) of cases, including increased intensity signal of the myocardium in T2, thinning of the LV, pericardial effusion, reduced LV and RV ejection fractions, LV diastolic dysfunction and kinetic abnormalities, and myocardial delayed contrast enhancement^[10]. Echocardiographic signs of heart abnormalities were also observed in 48% (25/52) of these patients, which underlines the superior sensitivity of the cardiac MRI modality. Cardiac MRI can be used to diagnose both structural and functional pathologies, such as myocardial inflammation and fibrosis (the extent of fibrosis and viable tissue is properly measured after contrast enhancement). The method gives an opportunity for quantitative assessment of myocardial perfusion and the effect of vasodilators^[3,10].

TDI also demonstrates an increased proportion of LV abnormalities in SSc patients. In 101 SSc patients, conventional echocardiography detected low LV ejection fraction (< 55%) in 7% (7/100) of patients, which was doubled to 14% (14/100) by use of TDI^[11-14].

Appearance of exercise-related changes should also be taken into account. Thus, using radionuclide ventriculography in 19 SSc patients, a reduced LV ejection fraction has been detected in 10.5% (2/19) of patients at rest, while the values were abnormal in 36.8% (7/19) after exercise^[15].

Thallium scintigraphy is another sensitive technique that detects perfusion defects in more than 70% of SSc patients with and without clinically manifested myocardial involvement^[16].

Some studies have found that scleroderma-related cardiac manifestations occur in both diffuse and limited cutaneous forms of SSc, whereas according to others, the prevalence is greater in the diffuse cutaneous form of the disease. An association between the cardiac involvement and the presence of anti-topoisomerase, anti-U3RNP antibodies, rapidly evolving skin disease, and skeletal myopathy has been implicated^[1,17,18].

When clinically manifested, cardiac involvement is thought to be an important prognosis factor^[3,19].

Myocardial involvement

The general pathogenetic mechanisms in SSc, including microvascular alterations (vasospastic episodes that are functional in the beginning with subsequent morphological vascular damage), collagen accumulation by activated

Table 1 Features of myocardial involvement in systemic sclerosis distinct from coronary atherosclerotic disease

Characteristic features
Microvascular ischemia
Patchy fibrosis, unrelated to coronary epicardial artery distribution
Involvement of immediate subendocardium, which is spared in atherosclerosis
Contraction band necrosis
Concentric intimal hypertrophy associated with fibrinoid necrosis of intramural coronary arteries
Hemosiderin deposits are not typically seen; they are evident in the atherosclerotic process

fibroblasts, and complex immune disturbances, are also thought to be involved in the pathogenesis of myocardial heart involvement in SSc^[3,20]. The ischemic, fibrotic and inflammatory lesions, which develop as a result of the above-mentioned processes, may also affect the conduction system, the pericardium, and the endocardium.

The consequences of the pathogenetic processes in SSc at the myocardial level result in areas of focal ischemia, recurrent ischemia-reperfusion injury, myocarditis and myocardial fibrosis. Microvascular alterations, but not the traditional atherosclerotic coronary disease, are thought to play a major role in the development of myocardial blood flow disturbances in SSc. Of note, myocardial infarction has been described in SSc patients with unaltered coronary arteries. Vasospasm of the small coronary arteries and arterioles (the so-called myocardial Raynaud's phenomenon) is considered to be involved in the early scleroderma-related ischemic myocardial changes with subsequent ischemia reperfusion injury and the development of structural vascular alterations. The early functional and reversible abnormalities have been demonstrated with thallium-201 SPECT at rest, after exercise and cold stimulation, PET, and cardiac MRI. In addition, an impaired vasodilator reserve has been found in SSc after causing maximum vasodilation with intravenous dipyridamol^[1,21].

A "mosaic", "patchy" distribution of myocardial fibrosis is a pathognomonic feature of the disease. In addition, foci of contraction band necrosis are typically found in all parts of the myocardium, including the immediate subendocardial area, which is usually spared in atherosclerotic processes^[22,23] (Table 1). At histological examination, distinct differences between myocardial fibrosis due to SSc itself and fibrosis in the context of coronary artery disease have been noted^[20]. In scleroderma-related heart involvement, the fibrotic areas do not correlate with pathologic changes of a single coronary artery. Hemosiderin myocardial deposits that are typically seen in atherosclerosis are absent in SSc-related myocardial pathology.

The inflammatory and autoimmune nature of SSc, as well as its possible association with skeletal myopathy, suggests that myocardial inflammation may play a crucial role in SSc heart disease. Myocarditis has been occasionally reported in SSc patients with acute and severe

cardiac symptoms^[21,24]. Interestingly, in a cohort of 181 SSc patients, a recent-onset heart disease was registered in 7 patients who underwent extensive noninvasive and invasive evaluations, including MRI and endomyocardial biopsy^[21]. Strikingly, all SSc patients with newly developed symptoms and signs of cardiac involvement were found to have biopsy-proven myocarditis. Administration of immunosuppressors (corticosteroids, cyclophosphamide, azathioprine) led to significant clinical improvement, normalization of cardiac enzymes, and improvement of MRI findings in nearly all cases.

Of note, a slightly increased thickness of the septum and posterior wall, or asymmetric septal hypertrophy have been found in a significantly higher number of SSc patients, including those without systemic arterial hypertension, as compared with healthy controls. Septal hypertrophy has also been observed secondary to PAH, which is often subclinical^[1].

The main clinical consequence of myocardial lesions is diastolic LV dysfunction, and less frequently systolic dysfunction, which both may be asymptomatic. In addition, different forms of atrial and ventricular arrhythmias, as well as symptomatic heart failure, may occur^[1,20].

Treatment

The administration of vasodilators, such as calcium channel blockers and angiotensin converting enzyme (ACE) inhibitors, has demonstrated beneficial effects on myocardial perfusion and limiting further progression of life-threatening complications^[5]. Improved myocardial perfusion and function in SSc patients with microvascular coronary pathology has been observed after treatment with nifedipine, nicardipine, or bosentan, using sensitive tests for evaluation such as cardiac MRI, TDI, and radionuclide ventriculography^[1,25-27]. Improved myocardial perfusion in scleroderma myocardial disease has also been found after treatment with ACE inhibitors^[28]. It has been hypothesized that there is a concomitant presence of ischaemic lesions accessible to reperfusion after vasospasm of small coronary vessels and irreversible lesions, such as morphological vessel pathology or myocardial fibrosis^[1]. Thus, administration of vasodilators may influence the reversible component of myocardial ischaemia.

A significantly lower number of SSc patients with reduced LV ejection fraction were found to have been previously treated with calcium channel blockers^[9]. These observations suggest that calcium channel blockers may protect against microvascular complications. Currently, dihydropyridine calcium channel blockers are the most well-studied and validated option for clinically apparent myocardial disease in SSc, and may be considered also for protective therapy. They have minimal negative inotropic effects and are generally well-tolerated, with reflex tachycardia and lower extremity edema being the most frequent side effects. Thus, they are recommended for regular administration in SSc-related myocardial disease unless contraindicated. In cases with concomitant PAH, they should be used with caution as they may lead to se-

vere systemic hypotension^[1].

Atherosclerosis

Accelerated atherosclerosis with increased cardiovascular morbidity and mortality is a well-known complication of many systemic inflammatory diseases that cannot be explained by the traditional cardiovascular risk factors. The hyperactivation of the immune system and systemic inflammation lead to premature atherosclerosis and earlier occurrence of its clinical manifestations. Thus, ischemic heart disease secondary to coronary atherosclerosis is the first cause of cardiovascular mortality in rheumatoid arthritis patients. Late mortality in systemic lupus erythematosus patients is mainly related to atherosclerotic disease, while in early phases, intercurrent infections are the leading cause^[29].

There are contradictory reports regarding the prevalence of atherosclerosis in SSc^[30]. According to some authors the prevalence of atherosclerosis of the large epicardial coronary arteries is similar to that of general population^[31]. In an autopsy study comparing 58 SSc cases to 58 controls, a significantly higher prevalence of ischaemic heart disease was found in the SSc patients^[32]. The frequency of epicardial vessel coronary atherosclerosis was similar (48% *vs* 43%), but atherosclerotic lesions of the small coronary arteries or arterioles occurred in 17% of SSc patients, compared with only 2% of controls. A study by Khurma *et al*^[33] comprised of 17 SSc patients and 17 healthy subjects that assessed the presence of coronary calcification by coronary CT, showed that signs of coronary atherosclerosis were present in 56.2% of SSc patients and in only 18.8% of age-, sex-, and race-matched controls.

Ho *et al*^[34] performed carotid duplex scanning and measurement of ankle brachial blood pressure index in 54 SSc patients and 43 control subjects that did not differ regarding cardiovascular risk factors. Their results showed that 64% of SSc patients had carotid artery disease compared with only 35% of the controls. In addition, SSc patients had a significantly higher prevalence (17%) of peripheral arterial disease. The results led to the conclusion that macrovascular disease is more common in SSc patient population. In addition, the mean intima media thickness, which is an indicator for the presence of atherosclerotic disease, has been shown to be either increased in SSc patients^[35] or unchanged^[36] as compared with healthy individuals.

The development of accelerated atherosclerosis in SSc is thought to be influenced by viral agents, immune reactions, anti-endothelial antibodies, or ischemia-reperfusion injury. Increased levels of C-reactive protein, homocysteine, von Willebrand factor, and vascular adhesion molecules, which are associated with the atherosclerotic process, as well as elevated and normal levels of lipids, have been reported in SSc^[29,37].

In a systematic review and meta-analysis of the literature, Au *et al*^[38] concluded that SSc patients are at an increased risk for atherosclerotic disease as compared

with healthy subjects. Microvascular disease is a pathognomonic feature of SSc as both vasospasm and structural alterations are present. Such alterations are thought to predict macrovascular atherosclerosis over time. However, the level of inflammation in SSc is lower than in rheumatoid arthritis and systemic lupus erythematosus. Thus, the atherosclerotic process may not be as aggressive and not easily detectable in small-number studies^[37].

Arrhythmias and conduction defects

Arrhythmias and conduction abnormalities are thought to be a result from conduction system fibrosis^[39,40] and myocardial fibrosis^[41]. Atrial and ventricular tachyarrhythmias result from myocardial fibrosis, whereas conduction defects and bradyarrhythmias are a consequence of conduction system fibrosis^[1].

Conduction system involvement is uncommon overall, rarely correlates with myocardial involvement, and is not usually clinically manifested^[39,40]. However, autopsy findings show that when fibrosis of the conduction tissues occurs, it most commonly affects the sinoatrial node^[39,40]. The most common clinical symptoms are dyspnea, palpitations, syncope. Of note, sudden death may also occur^[38].

At rest, normal electrocardiography has been recorded in over 50% of SSc patients, with an increase of arrhythmia rate noted during exercise^[41]. In 50 SSc patients, the most frequent abnormalities on the resting electrocardiogram were left anterior fascicular block (16%) and first-degree atrio-ventricular heart block (8%). The overall percentage of the abnormal findings was 32%. Of note, left bundle branch block and right bundle branch block with left anterior fascicular block were associated with abnormal left ventricular function, whereas isolated right bundle branch block or left anterior fascicular block were found in patients with normal left ventricular function^[41]. Twenty four-hour ambulatory continuous tape-recorded electrocardiograms demonstrated serious pathologic findings in a greater number of patients (62%): including supraventricular tachycardias (32%), conduction disturbances (14%), coupled ventricular extrasystoles (20%), and ventricular tachycardia (10%)^[42]. This same methodology also revealed conduction disturbances (such as sinus node dysfunction and first-degree heart block) and arrhythmias (*e.g.*, supraventricular tachycardia, atrial fibrillation, premature contractions from atrial or junctional origin, ventricular tachycardia, multifocal ventricular premature contractions) in 56.5% (26/46) of SSc patients^[43].

Supraventricular arrhythmias are considered to be more common in SSc patients, occurring in approximately two thirds of the cases, and much more frequent than ventricular tachyarrhythmias^[43]. Ferri *et al*^[44] also registered arrhythmias and conduction defects in a substantially higher proportion of SSc patients using 24-h Holter monitoring. In 53 SSc patients [34 with diffuse scleroderma and 19 with Calcinosis, Raynaud's phenomenon, Esophageal dysmotility, Telangiectasia syndrome (CREST)], rhythm and conduction abnormalities (*e.g.*,

conduction defects, supraventricular or ventricular arrhythmias and ST-T changes) were found in only 42% (22/53) on resting electrocardiogram. Using Holter monitoring, the number of detected conduction abnormalities increased from 10 to 16 patients, and transient ST-T changes increased from 2 to 18 patients. In addition, 48 patients had ventricular arrhythmias, with multiform ventricular premature beats in 21 (40%), pairs of runs of ventricular tachycardia in 15 (28%), and one or more runs of ventricular tachycardia in 7 (13%) cases. Furthermore, echocardiographic examination revealed asymmetric septal hypertrophy (10/53), impaired ventricular function (9/53), congestive cardiomyopathy (2/53), mitral prolapse (4/53), and pericardial effusion (3/53). Of note, multiform and/or repetitive ventricular premature beats occurred more frequently in patients with echocardiographic abnormalities, but were also present in patients who had normal findings on echocardiographic examination. It should be underlined, that the cardiac abnormalities did not correlate with the clinical variant of SSc (CREST syndrome or diffuse scleroderma), nor with other signs and symptoms of the disease.

Holter monitoring is therefore recommended in patients with symptoms of palpitations, lightheadedness, dizziness, or syncope, irrespective of the normal resting electrocardiogram. Exercise treadmill electrocardiogram may be helpful to identify exertional type arrhythmias. In all cases, a correlation with echocardiographic findings should be sought. Treatment protocols should follow the general guidelines in cardiology for management of the different forms of arrhythmias^[1].

Pericardial involvement

Pericardial abnormalities in SSc may manifest as fibrinous or fibrous pericarditis, pericardial adhesions, or pericardial effusion, and rarely as pericardial tamponade or constrictive pericarditis. Pericardial pathology is clinically apparent in over 5%-16% of the cases^[45]. The prevalence may be greater in SSc with limited cutaneous involvement (30%) *vs* patients with diffuse cutaneous form of the disease (16%)^[46]. At echocardiography, pericardial effusion can be detected in up to 41% of patients^[46], and in a larger proportion of cases (33%-72%) at autopsy^[45].

Pericardial involvement in SSc is usually clinically silent and benign. In the majority of cases, the presence of small pericardial effusion does not produce clinical symptoms and does not possess prognostic significance^[46]. Large hemodynamically significant pericardial effusions associated with heart failure may carry a poor prognosis and cause renal failure, probably due to the cortical renal hypoperfusion in the context of the large pericardial effusions and the administration of diuretics. Cardiac tamponade is rare and has a poor outcome. It should be emphasized that a small amount of rapidly accumulating pericardial fluid may cause tamponade because of the relative incapacity of the fibrotic pericardium for distension. Thus, close monitoring of SSc patients with acute pericarditis is recommended until complete resolution

of the symptoms, especially in the cases with coexisting myocardial involvement^[46]. Exudative pericarditis is easily diagnosed *via* echocardiography, which may be ordered after the findings on electrocardiogram (ST-T changes, low voltage) and chest X-ray (enlarged heart with a globular shape)^[46].

Pericardial effusions usually occur after the manifestations of other clinical features of SSc. Of note, large pericardial effusions, including those with development of tamponade, have been described prior to skin thickening and the establishment of the SSc diagnosis^[7,45,47,48]. Thus, SSc should be included in the diagnostic algorithm for the pericardial effusion of unknown origin. Pericardial effusions may also develop secondary to PAH or in the context of renal failure^[45].

Constrictive pericarditis presents as a right-sided heart failure with symptoms of shortness of breath, fatigue, anorexia, and wasting. Clinical manifestations may be in the context of both constrictive pericarditis and restriction due to myocardial fibrosis. Echocardiography, invasive measurement of LV and RV hemodynamic parameters, cardiac MRI and CT, may facilitate the differentiation. Diastolic septal bounce with increased respiratory variation in mitral inflow, discordance of peak left and right ventricular pressure at maximal inspiration, and enhanced and/or thickened pericardium on cardiac MRI, support the diagnosis of constrictive pericarditis. Of note, B-type natriuretic peptide (BNP) [and its cleavage product N-terminal pro BNP (NT-proBNP)], which is secreted from cardiomyocytes in response to atrial or ventricular wall stretch, and is increased in myocardial involvement, is normal or close to normal in constrictive pericarditis. Treatment includes diuretics, sodium and fluid restriction, and in selected cases, in the absence of contraindications (simultaneous presence of constrictive and restrictive pathologies and comorbidities), and pericardial stripping^[1].

The pathogenesis of pericardial effusion in SSc is thought to differ from rheumatoid arthritis and systemic lupus erythematosus. This notion is based on the findings that the pericardial fluid is noninflammatory by nature; auto-antibodies, immune complexes and complement depletion are absent. In addition, a general lack of response to corticosteroid treatment in scleroderma pericardial disease has been noted^[48]. At histological examination, nonspecific fibrotic pericardial thickening with adhesions and perivascular inflammatory cell infiltration have been found^[45].

Treatment

Treatment may include nonsteroidal anti-inflammatory drugs with close monitoring of renal function. Corticosteroids are considered to be of limited benefit in SSc-related pericardial disease^[45], but steroid-responsive cases also occur^[49], and corticosteroids may be life-saving in cases with associated myocarditis^[45]. Immunosuppressors may be indicated if profound inflammation is evident. Diuretics are considered in cases of heart failure but

should be used with caution due to the risk for development of renal failure^[45]. Pericardiocentesis is indicated in cases of life-threatening tamponade^[1].

SSc-related endocarditis

Valvular vegetations are considered to be rare manifestations in SSc. However, such lesions were found in 5 out of 28 autopsied SSc cases, including lesions of the mitral and tricuspid valve (alone or in combination), along with involvement of the chordae tendineae^[50], or the aortic valve^[51]. Nodular thickening of the mitral and aortic valves with regurgitation and mitral valve prolapse has also been noted^[45,52]. The clinical significance of such changes in SSc patients is unknown. Of note, endocarditis may occur in association with severe myocardial damage^[53].

Interestingly, embolisms in the brain and foot in SSc in the presence of mitral vegetation were found on echocardiography, and infective endocarditis was excluded on the basis of serial negative blood cultures and the absence of fever or known rheumatic valvular disease^[53].

SECONDARY CARDIAC COMPLICATIONS IN SSc

PAH

PAH is a syndrome resulting from restricted flow through the pulmonary arterial circulation with subsequent increased pulmonary vascular resistance and right heart failure^[54]. The prevalence of PAH in SSc is about 10%-12%^[55], varying between 4.9% and 26.7% depending on the applied diagnostic tools^[56]. PAH in SSc may be associated with pulmonary fibrosis, or may develop due to vascular narrowing or occlusion in cases with or without minimal pulmonary fibrosis. Pulmonary fibrosis is found in more than one third of SSc patients with either the diffuse or limited form of the disease. Post-mortem examinations revealed alveolar, interstitial, peribronchial and pleural fibrosis. PAH in the context of pulmonary fibrosis is usually of moderate degree and is characterized with relatively slow progression that develops as a result of the gradually increasing resistance of the pulmonary vasculature^[57,58]. PAH in SSc patients with minimal or no pulmonary fibrosis is a severe complication, and is a consequence of narrowing or occlusion of small pulmonary arteries caused by smooth muscle hypertrophy, intimal hyperplasia, vascular inflammation, and thrombosis *in situ*. Dyspnea from normal exercise tolerance to oxygen dependency progresses over 6-12 mo, with a mean survival of two years, whereas PAH in the context of lung fibrosis progresses more slowly, over two to ten years^[54,57,59,60].

Of note, isolated PAH, in the absence of pulmonary fibrosis, is more frequent in the limited cutaneous form of SSc (45%) than in the form with diffuse cutaneous involvement (26%)^[61]. Histological evidence for PAH at autopsy is also more frequent (over 65%-80% of cases). These data suggest a substantial prevalence of mild and moderate forms of PAH in SSc^[54,62]. PAH is considered

to be one of the most important factors contributing to the increased morbidity and mortality in SSc^[63]. The high incidence and prevalence of PAH in SSc, its poor prognosis, and the efficacy of the new evidence-based treatment that improves survival, stimulated the recommendation of an obligatory regular screening of pulmonary arterial pressure (PAP) in SSc patients.

Clinical signs of PAH include dyspnea on exertion, fatigue, chest pain, dizziness, palpitations, and edema at the lower extremities. Upon physical examination, an accentuated pulmonary component of the second heart sound, gallop, and pansystolic murmur of tricuspid regurgitation may be found, as well as features of right heart failure in advanced cases^[56]. The chest X-ray and electrocardiogram may reveal signs suggestive of PAH, mainly in the later stages, such as an enlarged pulmonary artery, attenuation of peripheral pulmonary vascular markings (at the chest X-ray), and peaked P wave ≥ 2.5 mm in leads II, III and aVF^[54,56]. If PAH is suspected, a transthoracic Doppler echocardiography is recommended^[54,56]. At echocardiography, PAH is defined as mean PAP > 25 mmHg at rest, > 30 mmHg during exercise, or systolic pulmonary pressure > 40 mmHg. Clues to diagnosis can be an elevated tricuspid regurgitation velocity (TRV) jet above 2.8 m/s, or a dilated right ventricle or atrium^[64]. The decreasing carbon monoxide diffusing capacity (DL_{co}) is a marker of pulmonary vascular disease and is standardly used in the diagnostic approach when PAH is suspected. Of note, it is associated with poor prognosis. CT and MRI may also be used to assess right ventricular mass, volume, and function. At MRI, the ratio of septal curvature, right ventricular ejection fraction, and right ventricular volume may be evaluated^[54].

All patients that are suspected of having PAH after noninvasive evaluation should undergo right heart catheterization (RHC) prior to therapy initiation. This method is the gold standard for diagnosing PAH, and allows for the measurement of the transpulmonary gradient (PAP mean wedge), which was found to be significantly elevated only in PAH patients, but not in patients whose pulmonary hypertension was due to increased cardiac output, left heart myocardial or valvular disease^[54,65]. A more reliable diagnostic parameter for PAH is pulmonary vascular resistance (PVR), which reflects the influence of transpulmonary gradient and cardiac output and is only elevated if the vascular obstruction occurs within the pre-capillary pulmonary circulation. However, PVR can also be elevated in patients with valve disease or left ventricular heart disease^[56]. Consequently, PAH is a diagnosis of exclusion. In the absence of lung disease, thromboembolism, left ventricular or valve pathology, the diagnosis of PAH requires both a mean PAP greater than 25 mmHg and a PVR greater than 3 Wood units with a pulmonary capillary wedge pressure < 15 mmHg (for exclusion of left heart disease)^[54,65]. In addition, BNP and NT-pro-BNP are promising screening parameters in SSc-related PAH, as increased levels correlate with disease severity

Table 2 Therapies for systemic sclerosis-related pulmonary arterial hypertension^[64-82]

Therapeutic approach	Dosage/comments
Prostanoids	
Epoprostenol: a prostacyclin with a very short half-life of 6 min; unstable at pH values below 10.5, requires intravenous administration ^[54,68]	Starting dose is 1-2 ng/kg per minute, gradually increased up to 25-40 ng/kg per minute
Treprostinil: an epoprostenol analogue with a half-life of 4.5 h, given as a continuous subcutaneous or intravenous infusion in patients with PAH from functional class II, III and IV ^[54,69]	10-20 ng/kg per minute
Iloprost: a chemically stable prostacyclin analogue with a longer half-life (20-25 min), given as a continuous intravenous infusion for 6-8 h ^[70]	0.5-3.0 ng/kg per minute
Beraprost: the first oral prostacyclin analogue with vasodilative and antiplatelet action and a half-life of approximately 1 h, indicated in primary and secondary PAH ^[72,73]	20 µg qid, may be increased by 20 µg/wk. The maximum allowed dose was 120 µg qid with a mean of 80 µg qid
Prostaglandins for inhalation	
Iloprost: inhalation has a pulmonary vasodilative potency similar to prostacyclin with longer effects (30-90 vs 15 min); effective in patients with severe PAH functional class III and IV ^[71]	2.5 or 5.0 mg six or nine times/d; median inhaled dose, 30 µg/d
Endothelin receptor antagonists	
Bosentan: the first drug from this group that was approved for treatment of PAH associated with systemic rheumatic diseases in the United States, Canada, Switzerland and European Union; indicated for PAH functional classes II, III and IV ^[74,75]	62.5 mg bid for 4 wk before titration up to 125-250 mg bid
Sitaxsentan: highly selective endothelin receptor antagonist with a long duration of action; high specificity for type A over type B receptors (6500:1) leads to blockade of the vasoconstrictory effect of endothelin-1 and maintenance of the vasodilative and clearance function of type B receptors ^[76]	50-100 mg/d
Ambrisentan: antagonist selective for type A over type B endothelin receptors (4000:1) ^[77]	2.5-10 mg
PDE inhibitors	
(PDE degrades cGMP, which mediates the effect of nitric oxide-a potent vasodilator and an inhibitor of platelet activation and vascular smooth muscle)	
Sildenafil: a specific inhibitor of the PDE-5 isoform present in large amounts in the lung ^[78]	20 mg 3 tid
Vardenafil: a PDE-5 inhibitor ^[80]	20 mg 3 tid
Tadalafil: a specific inhibitor of PDE-5 with a longer half-life (17.5 vs 3.8 h for sildenafil) ^[79]	20 mg 3 tid
Combination therapy	
(oral with inhaled and intravenous drugs)	
Sildenafil with intravenous epoprostenol Sildenafil and bosentan ^[81]	
Others	
Sodium consumption needs to be restricted to 2400 mg/d in patients with right ventricular failure; digoxin and diuretics when indicated	Saturation < 90% at rest or with exercise; Titration to an international normalized ratio of 1.5-2.5
Surgical options: atrial septostomy, single and double lung transplantation and combined heart and lung transplantation are ultimate therapeutic options in patients with end-stage disease ^[54]	
Routine immunization against influenza and pneumococcal pneumonia	
Oxygen therapy	
Anticoagulation therapy: (warfarin) in advanced stages with continuous intravenous therapy and in the absence of contraindications	
Although inflammation plays a significant role in the development and the progression of PAH, immunosuppression is not a common treatment, as systemic sclerosis-PAH is usually quite refractory to immunosuppressive drugs ^[82] . However, immunosuppressive treatment has led to improvements in some cases of PAH in other connective tissue diseases (e.g., systemic lupus erythematosus, primary Sjögren syndrome)	

PDE: Phosphodiesterase; PAH: Pulmonary arterial hypertension.

and predict survival^[54,64,65].

Treatment

The general therapeutic algorithm in SSc-PAH is summarized in Table 2. During RHC, vasodilator testing is performed in order to predict the therapeutic response. The response is defined as a reduction ≥ 10 mmHg to a mean PAP ≤ 40 mmHg, without a decrease in cardiac output^[54]. It includes administration of inhaled nitric oxide, intravenous epoprostenol or intravenous adenosine. It has been found that responders are more likely to have a sustained beneficial response to oral calcium channel blockers (long-acting nifedipine, diltiazem and amlodipine) than non-responders^[54,83-85]. Verapamil should be avoided because of its potential for negative inotropic

effects. High doses of calcium-channel blockers may improve survival in patients with primary PAH who respond with reductions in pulmonary arterial pressure and vascular resistance^[86].

SSc-associated PAH historically had a poor prognosis with a one-year survival rate of 45%^[55,87]. Survival, though still poor, has significantly increased with modern therapies such as prostanoids, endothelin receptor antagonists, and phosphodiesterase-5 inhibitors, which can improve pulmonary hemodynamics and functional capacity in patients with PAH in the context of connective tissue diseases. A six-year follow-up (2001-2006) of 315 patients with SSc-related PAH from the United Kingdom National registry has demonstrated one-, two- and three-year survival rates of 78%, 58% and 47%, respectively^[55,88,89].

Table 3 Criteria for definition of hypertensive scleroderma renal crisis

In the presence of limited or diffuse cutaneous scleroderma renal crisis:

A new onset of blood pressure > 150/85 mmHg obtained at least twice over a 24 h period. This blood pressure is defined as significant hypertension by the New York Heart Association

A documented decrease in renal function as defined by a decrement of at least 30% in the calculated glomerular filtration rate. When possible, initial results should be confirmed by a repeat serum creatinine concentration and recalculation of the glomerular filtration rate

To corroborate further the occurrence of acute renal crisis, it would be desirable to have any of the following (if available):

Microangiopathic hemolytic anemia on blood smear

Retinopathy typical of acute hypertensive crisis

New onset of urinary red blood cells (excluding other causes)

Flash pulmonary edema

Oliguria or anuria

Renal biopsy showing characteristic changes

Renal biopsy showing an alternative cause excludes the case from classification as scleroderma renal crisis

Early diagnosis of SSc-PAH and early subsequent intervention are essential for delaying disease progression. Early detection of PAH, when patients have few or no symptoms (*i.e.*, functional class I and II), is challenging. Available data broadly support annual screening of all SSc patients with and without symptoms. Patients with SSc who are at high risk for development of PAH are those with DL_{CO} < 60% predicted or who have declining DL_{CO} (*e.g.*, 20% decrease over a one-year period). Doppler echocardiography conducted at rest is considered to be the method of choice for PAH screening. For patients with TRV > 3.4 m/s (corresponding to a systolic PAP > 50 mmHg) or with a TRV between 2.9 and 3.4 m/s (corresponding to a systolic PAP between 34 and 49 mmHg) in the presence of other signs suggestive of PAH, non-invasive workup is recommended, including biomarkers, high-resolution CT and decision for confirmation of PAH *via* RHC^[90,91]. In the recently performed DETECT study aimed to define recommendations for earlier detection of SSc-related PAH, six variables were determined to guide to the echocardiography, including forced vital capacity/DL_{CO} (% predicted), presence of current/past telangiectasias, serum anticentromere antibodies, serum NT-proBNP, serum urate and right-axis deviation on electrocardiogram. TRV and right atrium area are evaluated in order to define the necessity of RHC for confirmation of PAH. It has been postulated that using TRV alone would fail to diagnose 20% of PAH patients when using a PAH suspicion threshold of ≥ 2.5 m/s, 36% when using a threshold of > 2.8 m/s, and 63% when using a threshold of > 3.4 m/s^[92].

Cardiac symptoms in severe scleroderma-related kidney involvement

A severe systemic hypertension due to SRC may trigger the development of systolic dysfunction and congestive heart failure^[4]. SRC occurs in over 10% of SSc patients, in 10%-25% in the subgroup of SSc patients with diffuse cutaneous involvement, and in only 1%-2% of those with a limited form of the disease^[1,93]. Most patients have a profound elevation of blood pressure at the onset of SRC; 90% have a pressure > 150/90 mmHg, and 30% have a diastolic pressure > 120 mmHg. Of note, an increase of 20 mmHg in blood pressure values may

be significant for a particular patient even though the values may still be in the normal range, and this also may represent a renal crisis. Only 10% of SRC are clinically associated with normal values of blood pressure. SRC is the most important renal complication in SSc based on vasculopathy, fibrosis and autoimmunity with a central role of the renin-angiotensin axis, as demonstrated by the striking clinical efficacy of ACE inhibitors^[1]. The diagnosis of hypertensive SRC is established on the basis of recently developed criteria (Table 3)^[94]. Factors for predicting the development of SRC include diffuse cutaneous involvement, rapid progression of skin involvement, disease symptoms for less than four years, presence of anti-RNA polymerase III antibody, new anemia, new cardiac events (pericardial effusion, congestive heart failure), and antecedent high-dose corticosteroids. Of note, previous blood pressure elevations, stable, mildly elevated serum creatinine, abnormal urine analysis, anti-topoisomerase and anticentromere antibodies, or pathologic findings in renal blood vessels are not predictive for the development of SRC^[1]. The clinical features include headache, breathlessness, dizziness, syncope. All SSc patients should be encouraged to check their blood pressure if such clinical symptoms are present. It is recommended that the SSc patients with predictive factors for the development of SRC should monitor their blood pressure twice weekly^[1].

Hypertension that occurs prior to the onset of SSc is usually essential, while those that develop after the onset of the disease could be either essential hypertension or, more likely, SSc-related^[95]. ACE inhibition is the cornerstone for the treatment of hypertension in SRC. ACE inhibitors should immediately be started once the diagnosis is established, or the dose increased if the patient is already taking them. ACE inhibitor resistance is a frequent finding in SSc patients. In such cases, the dose must be gradually increased to a maximum level. Early reduction or discontinuation of ACE inhibitors should be avoided. Denton *et al*^[96] recommend doubling the dose every 24 h, though deterioration of renal function may continue in this period. Frequently, it takes several days for blood pressure to fall to normal. In cases of insufficient blood pressure decrease, the authors recommend adding angiotensin receptor blockers, calcium channel blockers, doxazosin or clonidine^[96]. Beta blockers are contraindicated in

SRC due to their effect on peripheral circulation. Parenteral antihypertensives are not generally recommended, although nitrate infusion is sometimes indicated for the management of pulmonary edema^[1]. The aim of the antihypertensive treatment is to achieve pre-SRC values of blood pressure. The mean decrease per 24 h should be over 20 mmHg for the systolic and 10 mmHg for the diastolic blood pressure. Prolonged periods of hypotension should be avoided^[96]. If renal replacement is required, hemofiltration and hemodialysis are used depending on the hemodynamic stability and availability of the center. More than half of the patients who have undergone dialysis were able to discontinue it in 3-18 mo. Over 20% of the cases require chronic dialysis and 20% had an early death^[1].

SRC has been a leading cause for increased mortality in SSc, though survival has dramatically improved with the use of ACE inhibitors. Patients with SRC who received ACE inhibitors had an impressive one-year survival of 76% and a five-year survival of 65%, compared with 15% one-year survival and 10% five-year survival of patients not receiving ACE inhibitors, despite other aggressive antihypertensive treatment^[97,98]. The use of ACE inhibitors should continue indefinitely because recurrences occur years after the initial event when ACE inhibitors are discontinued^[1]. Prophylactic use of ACE inhibitors prior to SRC does not prevent its development^[1,99].

EVALUATION OF SSc PATIENTS WITH CARDIAC INVOLVEMENT

Laboratory investigations

Screening for biologic markers of possible cardiac dysfunction may be beneficial. One such laboratory marker is BNP, which is secreted from cardiomyocytes in response to atrial or ventricular wall stretch. Annual measurement of BNP is thought to be beneficial, as plasma concentrations correlate with the risk of death and cardiovascular events. BNP originates from the precursor protein pre-proBNP, which is first cleaved to proBNP, and then to active BNP and NT-proBNP. Both BNP and NT-proBNP can be measured in clinical practice, but the advantages of the latter are its longer half-life and increased stability. Of note, their levels vary according to gender and age. These markers are used for the screening of overall cardiovascular pathology in SSc, including PAH. There is not sufficient evidence that one natriuretic peptide is superior to another in this regard. The upper normal limits are 125 pg/mL for NT-proBNP and 60 pg/mL for BNP^[1,13,100,101]. Levels may be elevated in primary scleroderma-related myocardial involvement, as well as in pulmonary or systemic hypertension and in conventional concomitant cardiac diseases, such as acute and chronic coronary artery disease, left and right ventricular systolic and diastolic dysfunction, valvular heart disease, atrial arrhythmias, and heart failure^[1,13].

It should be emphasized that NT-proBNP is not cleared by natriuretic peptide clearance receptors and is

primarily excreted by the kidney. Thus, renal dysfunction is more likely to cause its elevation with less effect on BNP level. Of note, a number of noncardiac conditions may increase the level of natriuretic peptides, such as older age, female gender, weight loss, renal insufficiency, sepsis, pulmonary embolism, anemia, cirrhosis, corticosteroid administration, hyperthyroidism, malignancies, or central nervous system injury. On the other hand, factors such as obesity, constrictive pericarditis, pulmonary edema, and some cardiac medications (ACE inhibitors, angiotensin receptor blockers, beta blockers, diuretics, spironolactone) are associated with normal or decreased natriuretic peptide levels^[1].

Other laboratory markers that have been investigated together with NT-pro-BNP to evaluate a subclinical cardiac involvement in SSc and have shown significantly higher levels as compared with controls, are ischaemia modified albumin, high-sensitivity C-reactive protein, and Erythrocyte Sedimentation Rate. No significant differences have been detected for ischemia modified albumin and NT-pro-BNP levels between the limited and diffuse cutaneous forms of SSc. Ischaemia modified albumin is thought to appear in different conditions of local or generalized hypoxia and thus is not a specific cardiac marker^[101].

Troponin has not been found to be elevated in SSc despite myocyte loss and myocardial fibrosis. Thus, when elevated troponin is present, myopericarditis or non-scleroderma cardiovascular disease, such as coronary syndrome or pulmonary embolism, should be suspected^[1,101].

Instrumental investigations

A resting electrocardiogram is not sufficient to diagnose rhythm and conduction disturbances in SSc. Thus, when clinical signs like palpitations or syncope are present, 24-h Holter monitoring is indicated. Holter monitoring has demonstrated good sensitivity to detect arrhythmias and conduction abnormalities in a significantly higher percentage of patients as compared with the resting electrocardiography^[44], and should be included in the diagnostic algorithm of SSc patients with symptoms of palpitations, syncope, or dyspnea with unknown origin. New devices of Holter electrocardiographs may collect data for up to 14 d. In patients with less frequent symptoms, long-term Holter assessment (usually for a 30-d period) may be necessary. Of note, there are implantable monitors, which can detect arrhythmias indefinitely and may be also used in difficult cases^[1]. Exercise treadmill electrocardiogram may be helpful to identify the exertional type of arrhythmia^[1].

A transthoracic echocardiography should be included in the routine diagnostic screening of SSc patients^[1,44]. Of note, normal electrocardiographic findings were associated with normal left ventricular function at rest^[40]. Echocardiography allows measurement of atrial and ventricular dimensions, volumes (including ejection fraction), diagnosing of systolic and diastolic dysfunction, pericardial, valvular disease, and pulmonary hypertension^[1]. TDI

is a modern echographic method that allows the accurate measurement of regional and global LV and RV function, and the inclusion of this technique has improved the accuracy and reproducibility of standard echocardiography^[11].

Nuclear imaging, such as thallium-201 SPECT and PET scanning, are sensitive tools for detection of microvascular abnormalities in SSc-related myocardial disease. Detection of subendocardial ischemia by nuclear imaging is limited and inferior as compared with cardiac MRI^[1]. Cardiac MRI with or without contrast enhancement is a modern imaging modality that detects both structural and functional cardiac abnormalities in SSc patients with significantly superior sensitivity as compared with echocardiography (75% *vs* 48% detection rate in a cohort of 53 SSc patients)^[10]. Cardiac MRI gives an opportunity for quantitative assessment of myocardial perfusion and the effect of vasodilators^[3]. Chest CT may be used for combined assessment of lung and cardiac involvement in SSc. Cardiac CT and MRI are valuable techniques for detection of pericardial thickness and inflammation^[1].

Cardiac catheterization is indicated in SSc for diagnosis of PAH, constrictive pericarditis, cardiac tamponade and epicardial coronary artery disease, for performing endomyocardial biopsy in cases of suspected infiltrative cardiac disease^[1].

CONCLUSION

Cardiac involvement in SSc may present with various manifestations and is an indicator of a poor prognosis. The rheumatologists should be acquainted with the different forms of primary and secondary cardiac involvement in SSc and the necessity for screening for the detection of subclinical cases *via* modern sensitive tools, as early diagnosis and treatment are crucial for a positive outcome.

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Serum creatinine role in predicting outcome after cardiac surgery beyond acute kidney injury

Mahdi Najafi

Mahdi Najafi, Tehran Heart Center, Tehran University of Medical Sciences, Tehran 1411713138, Iran

Author contributions: Najafi M solely contributed to this paper.
Correspondence to: Mahdi Najafi, MD, Tehran Heart Center, Tehran University of Medical Sciences, North Karegar Ave, Tehran 1411713138, Iran. najafik@sina.tums.ac.ir

Telephone: +98-21-88029674 Fax: +98-21-88029724

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and prediction of AKI, but also the most important predictor of outcome after cardiac surgery, including mortality and morbidity as well as hospital length of stay.

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Abstract

Serum creatinine is still the most important determinant in the assessment of perioperative renal function and in the prediction of adverse outcome in cardiac surgery. Many biomarkers have been studied to date; still, there is no surrogate for serum creatinine measurement in clinical practice because it is feasible and inexpensive. High levels of serum creatinine and its equivalents have been the most important preoperative risk factor for postoperative renal injury. Moreover, creatinine is the mainstay in predicting risk models and risk factor reduction has enhanced its importance in outcome prediction. The future perspective is the development of new definitions and novel tools for the early diagnosis of acute kidney injury largely based on serum creatinine and a panel of novel biomarkers.

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Key words: Creatinine; Acute kidney injury; Cardiac surgery; Outcome; Biomarker

Core tip: This manuscript aims to review the latest achievements in the diagnosis and treatment of acute kidney injury (AKI). Despite much progress in recent years, especially in the development of novel biomarkers, serum creatinine still plays the major role. Creatinine is not only the mainstay of definition, diagnosis

INTRODUCTION

Creatinine is an important determinant in cardiac surgery. Rise in the level of serum creatinine has a significant impact on surgical outcome. Acute kidney injury (AKI) is basically defined by perioperative changes in serum creatinine level. Even minimal changes in serum creatinine not high enough to be defined as AKI worsen the outcome of patients who undergo cardiac surgery. Sensitivity of serum creatinine is low and its response to renal insult is slow and late. However, serum creatinine level still constitutes the main measure for the assessment of renal function thanks to the simplicity and availability of its measurement. Similarly, serum creatinine is the cornerstone of the consensus definitions of AKI. Indeed, an acronym for Risk of renal failure, Injury to the kidney, Failure of kidney function, Loss of kidney function and End-stage renal failure (RIFLE), acute kidney injury network (AKIN) and Kidney Disease: Improving Global Outcomes (KDIGO) all use creatinine for grading the severity of AKI^[1,2]. The principal role of creatinine as a main predicting factor in the scoring systems for risk estimation is well known^[3]. Creatinine has, therefore, been included in the first three important risk factors for mortality after cardiac surgery by newer prediction scores^[4].

With little tolerance, we assume an abrupt rise in serum creatinine as acute kidney injury (AKI). Due to

the unique characteristics and specifications of AKI that occur after cardiac surgery, it has been called cardiac surgery associated AKI (CSA-AKI). In recent years, many investigations have been performed to find answers to key questions on the prevention and treatment of CSA-AKI in the perioperative period. Numerous studies have been performed and are underway with their focus on the CSA-AKI^[2,5] and there are promising results, especially in prophylactic management. However, recruitment of patients with minimum risk of AKI for clinical trials on CSA-AKI treatment is the main reason why most of these studies lack the sufficient power to be conclusive^[2,5]. Furthermore, inconsistency in the definition of AKI between different studies makes it difficult to analyze the results of these studies in meta-analyses^[5,6].

This review covers the following: (1) association of serum creatinine with cardiac surgery-associated mortality and morbidity; (2) serum creatinine role in diagnosis of cardiac surgery-associated acute kidney injury; (3) risk factors for high perioperative serum creatinine; (4) risk models for AKI after cardiac surgery; (5) creatinine and the outcome prediction in cardiac surgery; and (6) prevention and treatment on the horizon.

ASSOCIATION OF SERUM CREATININE WITH CARDIAC SURGERY-ASSOCIATED MORTALITY AND MORBIDITY

The development of postoperative AKI has been recognized as the strongest risk factor for death in patients undergoing cardiac surgery^[7]. It has been shown that AKI occurs in up to 40% of patients undergoing cardiac surgery^[2]. As much as the incidence is rare (1% to 5%), mortality among patients with AKI who require renal replacement therapy (RRT) or become dialysis dependent is more than 50% and approaches 80% in patients who need dialysis, while the overall mortality rate after cardiac surgery hardly exceeds 8%^[7-9].

AKI increases postoperative morbidity, length of stay in the intensive care unit (ICU) and hospital and costs of care^[10]. High level of preoperative serum creatinine is associated with higher risk of RRT and need for dialysis after cardiac surgery^[11,12]. Even minimal changes in serum creatinine increase postoperative mortality significantly. Indeed, 30 d mortality was reported to be 2.8 and 18.6 fold higher with up to a 0.5 mg/dL and more than 0.5 g/dL creatinine rise, respectively, compared to no change in a group of patients who underwent cardiac surgery^[13]. The risk of AKI increases in valvular and combined surgery compared to myocardial revascularization two to four times, respectively^[11,14,15].

It has been indicated repeatedly in different studies that serum creatinine rise after cardiac surgery is followed by long-term chronic kidney disease (CKD) and mortality^[16-19]. Moreover, higher degrees of preoperative kidney insufficiency are accompanied by a proportionally higher risk of CSA-AKI and need for RRT^[20]. Pathophysiologi-

cal studies indicate that cardiac patients with AKI are more likely to have progressive renal changes beyond the acute episode even after reduction of serum creatinine to normal levels^[19,21,22].

AKI has been divided into pre-renal, renal and post-renal with regards to etiology. In surgical patients, pre-renal etiology, followed by renal etiology, is the most common cause of AKI^[23]. As volume changes are common during cardiac surgery, CSA-AKI can be divided into volume responsive and non-volume responsive which usually matches pre-renal and renal etiologies. Renal etiology of CSA-AKI is caused by various factors, including ischemia and ischemia-reperfusion injury, inflammation and oxidative stress, exogenous and endogenous toxins, metabolic abnormalities and neurohormonal activation^[24]. They can briefly be divided into hemodynamic, inflammatory and nephrotoxic factors^[25,26].

SERUM CREATININE ROLE IN DIAGNOSIS OF CSA-AKI

In this section, we discuss that new equations have improved the calculation of glomerular filtration rate estimation (eGFR), especially in people with suboptimal kidney function and near normal real GFR. Moreover, new consensus systems are focused on more accurate practical definitions of AKI so that they can be better tools for outcome prediction. Nonetheless, there are obstacles to employing these formulas and definitions in cardiac surgery and creatinine still rules supreme.

Kidney impairment after cardiac surgery is acute in onset and most probably occurs in patients without a previous history of renal insufficiency. However, conventional formulas for eGFR have been released by studies on patients with renal impairment. Similarly, well-known definitions of AKI have been developed by analyzing data from patients with previous CKD. Paradoxically, most of the studies on AKI diagnosis and management after cardiac surgery have been performed in people without CKD, while we know CKD is the most important predictor of postoperative AKI. This shows how challenging it is to study the most important complication of cardiac surgery.

Creatinine clearance and eGFR methods

Serum creatinine has been used to determine GFR for a long time. Even the diagnosis and staging of CKD has been made through serum creatinine measurement. However, the serum concentration of creatinine is affected by factors such as age, gender, ethnicity, diet, muscle mass and medication. Moreover, creatinine will not be higher than the normal range until 50% of renal function is lost^[27]. Direct measurement of GFR with inulin or radio-nuclides is expensive and complex and thus not suitable for routine use. Furthermore, the older method of 24 h urine sampling for the measurement of creatinine clearance is not easy to perform and the results are biased owing to some tubular secretion of creatinine that causes

up to 40% overestimation of GFR compared to inulin clearance^[27]. That is why better tools for the assessment of GFR are required.

There are several creatinine-based formulas for the estimation of GFR that are widely used in research and clinical practice. The Cockcroft-Gault formula, named after the two scientists who developed it in 1976, is the most common surrogate for creatinine clearance in the estimation of GFR^[28]. This formula employs age and weight as well as gender to calculate eGFR. The formula is useful due to simplicity and ease of calculation and it underscores the importance of age in estimating GFR: for the same level of creatinine, eGFR decreases to half at age 80 compared to age 20. However, the use of this simple equation in obese patients is not possible. Accordingly, ideal body weight, which is calculated taking height into account, is utilized in this group of people. Similarly, using adjusted body weight again while considering the role of height improves the GFR estimation in the elderly^[29]. These shortcomings limit its application in the laboratory to report creatinine clearance.

Another common formula was developed by the Modification of Diet in Renal Disease (MDRD) Study Group in 1999^[30]. It was simplified in 2002 by omitting albumin and blood urea nitrogen to a 4-variable MDRD which estimates GFR using the variables of age, gender, race and serum creatinine^[31]. Laboratories use this formula to report eGFR. This formula estimates GFR more precisely in patients with CKD. Nevertheless, both 6-variable and 4-variable MDRDs underestimate GFR in healthy individuals with creatinine clearance more than 60 mL/min. Furthermore, compared to the Cockcroft-Gault equation, MDRD do not adjust for body mass index and thus underestimates GFR in obese and overestimates GFR in underweight people^[32,33].

The most important shortcoming of both Cockcroft-Gault and MDRD equations is their development in patients with CKD. What is more, it has been shown that both formulas have lower precision in people with normal GFR^[33-35]. Cockcroft-Gault overestimates and MDRD underestimates GFR in this group of healthy population^[36]. Renal insufficiency in cardiac surgery is acute in onset and most probably in patients without any history of CKD. These formulas may, therefore, not be as useful in this group of patients^[37]. To overcome this problem, the CKD-epidemiology collaboration (CKD-EPI) developed a new formula in 2009. This equation is superior to MDRD when GFR is more than 60 mL/min. Unlike the other two formulas, CKD-EPI was developed through several studies in populations with suboptimal renal function^[38]. Advantageous in the CKD-EPI equation is its probable improved cardiovascular risk prediction compared to MDRD in a middle-age population^[39]. Findings in previous studies will probably need revision through a new formula^[40].

Development of new equations and making modifications to the available ones reflect the attempts to make eGFR an ideal surrogate for real GFR as much as possible. However, the closer we get to an accurate estimate

of GFR, the farther we get from a clinically more practical tool. The most important drawback to employing these formulas in clinical practice is the fact that we cannot find a formula to fit all clinical conditions. Accuracy of eGFR is compromised when the clinical condition is different from the populations from which the equations were derived. Malnutrition or reduction in muscle mass from illness or amputation, extremes of muscle mass and diet (such as vegetarians), different ethnicities from those included in studies used for the development of the equations, or changes in the non-GFR determinants over time are the most probable determinants of large differences between real and estimated GFR^[27,34].

A newer concept is to add laboratory parameters that are not dependent on body muscle mass and nutrition so as to obtain a better estimation of GFR. Using cystatin C, an index of glomerular function, is believed to be promising in different investigations in adults and children^[34,41,42]. There are even equations based on cystatin C to calculate GFR^[42,43]. However, cystatin C needs adjustment for age, gender and race, although adjusted cystatin C is probably superior to adjusted creatinine in developed equations^[44]. Moreover, the lack of an international standard to calibrate cystatin C limits the use of these equations. More to the point, we do not know whether the routine use of cystatin C merits its cost as there is no evidence that it improves outcome significantly^[45].

Preoperative creatinine and occult renal insufficiency

Discussion on preoperative creatinine revolves around the most important risk factor for CSA-AKI which is previous renal insufficiency^[8,46]. Nevertheless, no unique level of serum creatinine as a threshold for renal insufficiency has been defined^[47]. Predictor models for AKI are also not consistent: although most of them have reported preoperative renal insufficiency as a risk factor, their definitions for renal insufficiency are largely diverse from baseline creatinine of 1.5 mg/dL and eGFR of 60 mL/min as cut off points for dialysis dependency^[48]. This holds true for most of the predictor models of outcome in cardiac surgery that have included preoperative renal insufficiency as a predictor^[3,4,49]. Proteinuria, the most prevalent parameter used in the definition of CKD, is also absent in most of them due to a lack of data on urinalysis^[5].

There is no debate on patients who are dialysis dependent or need renal replacement therapy. We recognize these problems as kidney disease. The most challenging are the patients whose serum creatinine level is within normal range but their real GFR or eGFR is low. We call this condition occult renal insufficiency and it is usually defined as eGFR < 60 mL/min when creatinine is in normal range. Several studies have shown that the incidence of morbidity and mortality after cardiac surgery is higher in patients with occult renal insufficiency^[50-53].

AKI definition systems: RIFLE, AKIN and KDIGO

Despite the recognized importance of AKI, one of the major problems in conducting studies on the subject is

Table 1 Definition and classification for acute kidney injury

	Serum creatinine/GFR criteria	Urine output criteria
RIFLE classification		
Definition	SCr rise ≥ 1.5 times baseline or GFR decrease $> 25\%$ within 7 d	
Staging	R (Risk) SCr rise up to 2 times baseline or GFR decrease $> 25\%$	< 0.5 mL/kg per hour for ≥ 6 h
	I (Injury) SCr rise up to 3 times baseline or GFR decrease $> 50\%$	< 0.5 mL/kg per hour for ≥ 12 h
	F (Failure) SCr rise 3 times baseline or more or GFR decrease $> 75\%$ or absolute SCr ≥ 4 mg/dL with acute rise ≥ 0.5 mg/dL	< 0.5 mL/kg per hour for ≥ 24 h or anuria ≥ 12 h
	L (Loss) persistent AKI > 4 wk, need for RRT	
	E (ESRD) persistent loss > 3 mo, need for dialysis	
AKIN classification		
Definition	SCr rise ≥ 1.5 times baseline or ≥ 0.3 mg/dL within 48 h	
Staging	1 SCr rise up to 2 times baseline or ≥ 0.3 mg/dL	< 0.5 mL/kg per hour for ≥ 6 h
	2 SCr rise up to 3 times baseline	< 0.5 mL/kg per hour for ≥ 12 h
	3 SCr rise 3 times baseline or more or absolute SCr ≥ 4 mg/dL with acute rise ≥ 0.5 mg/dL or need for RRT	< 0.3 mL/kg per hour for ≥ 24 h or anuria ≥ 12 h
KDIGO classification		
Definition	SCr rise ≥ 1.5 times baseline within seven days or ≥ 0.3 mg/dL within 48 h or oliguria	
Staging	1 SCr rise up to 2 times baseline or ≥ 0.3 mg/dL	
	2 SCr rise up to 3 times baseline	
	3 SCr rise 3 times baseline or more or absolute SCr ≥ 4 mg/dL with acute rise ≥ 0.5 mg/dL or need for RRT	

GFR: Glomerular filtration rate; AKIN: Acute Kidney Injury Network; KDIGO: Kidney Disease: Improving Global Outcomes; RIFLE: An acronym for Risk of renal failure, Injury to the kidney, Failure of kidney function, Loss of kidney function and End-stage renal failure; SCr: Serum creatinine; AKI: Acute kidney injury; RRT: Renal replacement therapy.

the lack of consensus regarding the diagnosis as there are more than 30 different definitions for AKI^[47]. In the past decade, several consensus systems have been introduced to define AKI uniformly in different studies. Perioperative changes in the serum concentration of creatinine are the cornerstone of the definition in these systems. In 2004, the RIFLE criteria were proposed by the Acute Dialysis Quality Initiative group^[47].

A revised version of the RIFLE criteria was suggested by the AKIN group in 2007. There are four main changes in AKIN compared to RIFLE (Table 1): GFR changes have been omitted from the definition system; time period of seven days for creatinine changes has been replaced by 48 h; creatinine changes as low as 0.3 mg/dL is the lowest measure to be considered as AKI; and the two outcome determinants in RIFLE (loss and end stage) are deleted to define AKI in three stages^[54].

Following the establishment of the AKIN scoring

system, the resultant debate over the supremacy of each criterion prompted comparative research^[55-60], which disclosed that AKIN was not more efficient than RIFLE and that some authors still preferred to employ RIFLE with some modifications^[55]. Modified RIFLE stages anyone who needs RRT in category F (failure) regardless of the level of serum creatinine. A recent study performed with this method reported an incidence of 14% for AKI and showed that CSA-AKI aggravated short and long term outcomes in cardiac patients^[19]. Nevertheless, a large survey of 1881 patients by Bastin *et al*^[61] indicated that the incidence of AKI with both AKIN and RIFLE criteria was mostly equal (25.9% and 24.9%, respectively), but hospital mortality was predicted more precisely by AKIN. Another dispute was over the sensitivity of the two definitions insofar as whether or not the designated thresholds sufficiently diagnose all the cases of renal impairment. Studies have shown that there are concerns about the adequacy of the AKIN and RIFLE criteria inasmuch as that by the current standards, some AKI cases may be left undiagnosed. Lassnigg *et al*^[62] described a new scoring system and reported that determining the amount of serum creatinine changes within 48 h was more capable than the RIFLE or AKIN criteria in predicting post-surgical outcomes.

This idea and the results of other studies encouraged researchers to propose a new definition. The KDIGO workgroup has recently reviewed these criteria and published a single definition for use in both clinical practice and research. AKI is defined when any of the following three criteria are met: an increase in serum creatinine by 50% in seven days, an increase in serum creatinine greater than 0.3 mg/dL in 48 h or oliguria^[1]. There is a paucity of data to judge KDIGO as few studies have employed this criterion to date^[63,64]. However, AKI incidence using KDIGO definition is probably lower than that using AKIN and RIFLE. Reported incidences of AKI in different studies ranged from 26%-49% for AKIN^[55,60], 19%-30% for RIFLE^[55,60] and 15%-16% for KDIGO^[63,64].

Thanks to the development of consensus systems for the definition of AKI, it is possible currently to compare studies around the world and newer definitions have improved their employment in cardiac patients. Nevertheless, we are still far from an ideal practical definition of CSA-AKI. One reason may be the effect of the minimal changes in creatinine on outcome. Although this has been investigated largely in patients undergoing cardiac surgery^[13,62], it is not limited to cardiac patients^[65]. The AKIN definition sets a lower minimum level of serum creatinine as the diagnosis cut off point for AKI. However, even people who are outside of the minimum level have a worse outcome compared to patients with almost no change in serum creatinine. As employing the current systems for AKI definition in clinical practice is not easy, many of the studies performed to date have utilized these definitions partially. This is probably more pronounced in the RIFLE criteria which require seven days of follow-up

for the diagnosis to be completed^[66].

AKI biomarkers, creatinine as a biomarker

Conventional biomarkers: An ideal biomarker for AKI is noninvasive, specific and sensitive for the detection of AKI within 24 h and is detected and measured in a rapid and reproducible way. Moreover, it should stratify risk and identify AKI subtypes^[1,27,67,68]. A single biomarker that can fulfill all these criteria has yet to emerge^[69]. Serum creatinine as a biomarker is still the only reliable tool for the assessment of AKI. Urine output is readily available and more sensitive to hemodynamic changes compared to creatinine. However, its variations are not specific, especially during cardiac surgery with cardiopulmonary bypass (CPB), and unavoidable hemodynamic changes, due to medications, such as diuretics, mannitol and other fluids, and possible measures such as ultrafiltration. In addition, the well-known term of non-oliguric renal failure denotes that normal urine output does not guarantee normal renal function^[70,71]. The other marker, urinalysis, can differentiate pre-renal from renal failure in patients with decreased urine output which is very helpful in guiding treatment. Obviously urinalysis is not suitable for prophylactic measures due to its delayed response to renal insult^[71].

With regard to eGFR formulas, we know that there is a lag between the renal event and serum creatinine changes that may be as long as 48 h, while we expect to know the occurrence of renal impairment immediately after surgery. As creatinine is not a sensitive measure, GFR may decrease up to 50% before the creatinine starts to change. Moreover, as creatinine is not specific, its value is influenced by changes in age, gender, race and muscle mass, as discussed before. In cardiac surgery, changes in total body volume, protein intake and medications may extend the list^[27,72]. These factors are so important that, for instance, volume overload was reported to be superior to creatinine in predicting outcome after cardiac surgery in a recent study^[73].

Novel biomarkers: Using the most sensitive and specific biomarker for AKI is the ideal solution for the optimal estimation of GFR and rapid diagnosis of renal insult. As was noted, such a biomarker should be biologically stable and as a laboratory assay should be quick, reliable and cost effective with a high discriminative power^[67]. So important is this issue that finding a suitable biomarker was recommended as the key search area in 2005^[74]. Currently, two large studies are underway to assess the role of novel biomarkers in the diagnosis and prognosis of AKI: multicenter National Heart, Lung and Blood Institute-sponsored Translational Research Investigating Biomarker Endpoints in AKI (TRIBE-AKI) study and the Assessment, Serial Evaluation and Subsequent Sequelae of AKI study. The latter is also aimed at evaluation of long term complications of AKI^[75]. The results of these large studies are expected to shed sufficient light on the matter.

In recent years, more than 20 biomarkers have been introduced and most of them have been tested in studies of post-cardiac surgery^[68,76]. Four novel biomarkers have been studied most frequently: neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1) as markers of tubular injury and cystatin C as a marker of glomerular function. NGAL, followed by IL-18, is more promising as an early diagnostic tool and may qualify for entry into clinical practice. KIM-1 has delayed response and cystatin C needs adjustment for age, gender and race^[2,69,77].

NGAL: NGAL is a protein that normally binds to small iron-carrying molecules. NGAL is significantly upregulated in response to renal tubular injury. Role of NGAL in the diagnosis of AKI has been the most extensively studied in cardiac surgery^[78]. First, animal studies in 2003 showed that NGAL was markedly upregulated early after ischemic injury^[79]. Then its rapid rise following renal insult drew attention. Level of urinary NGAL one hour post-CPB significantly predicted the risk of AKI after cardiac surgery^[80]. Plasma NGAL levels two hours after CPB were strongly correlated with the duration and severity of AKI^[81]. Other studies showed that NGAL levels were predictive of CSA-AKI when measured both in urine and plasma^[82-85].

It is noteworthy that the predictive power of NGAL in pediatric surgery is striking, whereas its sensitivity and specificity for AKI prediction in adult cardiac surgery is not high enough to employ it as the sole biomarker for CSA-AKI^[78,86]. It shows that the nature of CSA-AKI in adults is probably more complex. Degrees of chronic renal impairment before cardiac surgery may explain part of this inconsistency between the response of biomarkers to renal insult in adults and children. It is evident from recent studies that the diagnostic performance of NGAL is significantly influenced by baseline renal function^[84,87].

IL-18: IL-18, a pro-inflammatory cytokine, is a biomarker of AKI and is detectable in urine four to six hours after CPB, peaking at 12 h^[88]. A multi-center study showed that plasma NGAL and urine IL-18 peaking within 6 h after cardiac surgery not only predicted AKI earlier than serum creatinine, but also predicted important outcomes such as length of stay in the ICU and hospital, dialysis and death^[89].

However, there are some challenges regarding the use of the currently available biomarkers. First, biomarkers are being evaluated in comparison with creatinine as a gold standard while the weakness of serum creatinine to be a sensitive and specific marker has been the main cause of directing research into finding novel biomarkers^[90]. Second, many of the studies undertaken to date have excluded patients with CKD^[70] while CKD is the most important risk factor for postoperative AKI. Discrepancy between clinical practice and the results of research may arise as biomarkers are under the influence of baseline renal function^[11,12]. Third, the level of bio-

Table 2 Risk factors for acute kidney injury

Preoperative	Intraoperative
Patient related	Patient related
Renal dysfunction/high SCr¹	Low venous compliance
Advanced age	Low systemic vascular resistance
Female gender	Autoregulatory systems disturbances
NYHA FC IV	Low output syndrome
Reduced LVEF or CHF	(pressor/IABP need)
Left main CAD	Type of surgery
Diabetes mellitus	Valvular
Poor glycemic control	Re do surgery
Peripheral vascular disease	Emergency
COPD	
Coexisting liver disease	
Preoperative IABP	
Pulmonary rales	
Genetic predisposition	
Modifiable	Procedure related ³
Extremes of SBP ²	On-pump cardiac surgery
Sepsis ²	Nonpulsatile flow on CPB
Medications (NSAID, ARB)	Hypothermic CPB
Contrast dye	Deep hypothermic circulatory arrest
	Duration of CPB (> 100-120 min)
	Perfusion pressure
	Hemodilution during CPB
	Blood transfusion
	Hemolysis
	Embolism

¹Risk factors with higher level of evidence are in bold; ²both patient related and modifiable; ³and also modifiable. NYHA FC IV: New York Heart Association Function class IV; LVEF: Left ventricle ejection fraction; CHF: Congestive heart failure; CAD: Coronary artery disease; COPD: Chronic obstructive pulmonary disease; IABP: Intra-aortic balloon pump; SBP: Systolic blood pressure; NSAID: Nonsteroidal anti-inflammatory drug; ARB: Angiotensin receptor blockers; CPB: Cardiopulmonary bypass; SCr: Serum creatinine.

markers increases in response to injury. Although novel biomarkers are superior due to earlier response, the ideal biomarker would be one that predicts AKI preoperatively. Promising results have been reported with ouabain^[91]. Fourth, the pathogenesis of AKI is multifactorial. Hemodynamic, inflammatory and nephrotoxic factors are responsible and overlap each other in leading to kidney injury^[25]. This complex pathology affects finding a unique biomarker with high accuracy in the diagnosis of AKI. Consequently, no biomarker by itself is an accurate and reliable predictor for the diagnosis and risk estimation in AKI. Combination of biomarkers as a diagnostic panel would probably allow the determination of the risk and severity, as well as the early diagnosis of AKI^[76,92].

RISK FACTORS FOR HIGH PERIOPERATIVE SERUM CREATININE

Risk factors for increased level of serum creatinine and the development of AKI have been widely studied^[10,71]. There are two main groups of risk factors: preoperative and intraoperative. Most of the preoperative risk factors are patient-related and most of the intraoperative risk factors are procedure-related. Usually, intraoperative risk

factors are more likely to be modifiable^[25] (Table 2). Post-operative factors, such as blood drainage and need for excessive transfusion and emergent exploration, as well as myocardial infarction, are of limited interest due to late onset and low chance of their benefit in AKI prediction and prevention^[10].

Preoperative risk factors

Preoperative risk factors are not the same in different studies. The most reported risk factors include advanced age, female gender, New York Heart Association Function class IV, reduced left ventricular ejection fraction or congestive heart failure, diabetes mellitus, poor glycemic control, peripheral vascular disease and chronic obstructive pulmonary disease. Other factors such as the need for preoperative intra-aortic balloon pump and pulmonary rales have been noted in studies. However, the most predictive risk factor has consistently been preoperative renal dysfunction^[2,71]. Thakar *et al*^[14] developed a risk index for predicting the need for dialysis after cardiac surgery based on preoperative factors. This study showed that the value of preoperative serum creatinine as an equivalent for renal dysfunction is the most important predictor for AKI.

Several studies have suggested that medications such as non-steroidal anti-inflammatory drugs and angiotensin receptor blockers (ARB) be stopped before cardiac surgery in order to decrease the risk of AKI^[25]. More recently, genetic predisposition to AKI has been studied. According to many polymorphism studies, apolipoprotein was associated with AKI and its epsilon-4 allele has been the only genotype protective against AKI compared to other forms of allele^[93,94].

Intraoperative risk factors

Contrary to many preoperative risk factors that are well known for their role in the development of CSA-AKI, the identification of intraoperative risk factors is challenging. Maintaining stable hemodynamics is probably the most important point in kidney protection during cardiac surgery, especially on the CPB. This is supported by the finding that many intraoperative risk factors are associated with hemodynamic instability: low-output syndrome; intraoperative intra-aortic balloon pump use; pressor need prior to CPB; and the need for deep hypothermic circulatory arrest. However, the management of hemodynamic changes is not easily feasible because patient factors such as venous compliance, systemic vascular resistance and autoregulatory systems are responsible for cardiovascular stability during cardiac surgery and are difficult to control^[10].

Rather than surgery type (valvular, re do, emergency), modifiable procedure-related risk factors include on-pump cardiac surgery, CPB nonpulsatile flow and hypothermic CPB. Current data is insufficient to confirm the association between these CPB parameters and the risk of CSA-AKI^[2,71]. Other more established CPB-related risk factors are duration of CPB (> 100-120 min), perfu-

sion pressure, hemodilution during CPB, blood transfusion, hemolysis, most commonly due to cardiotomy suction, and embolism^[2,95,96]. The role of CPB in inducing systemic inflammatory response syndrome (SIRS) and consequently CSA-AKI has been shown in different cardiac surgery events. The inflammation is related to perfusion pressure, hemodilution, blood transfusion, hypothermia, hemolysis and embolism^[97,98]. SIRS and other physiological untoward events explain how much longer CPB time increases the incidence of CSA-AKI. A meta-analysis in 2009 showed that mean CPB time and mean cross clamp time were significantly longer in patients who developed AKI. No safe time limit has been reported, however^[99].

Surgical technique

Surgical techniques with minimum CPB usage potentially lessen the adverse complications of the inflammatory response. Minimally invasive cardiac surgery, including transcatheter aortic valve implantation, or minimally invasive mitral valve surgery decreases the incidence of AKI^[100]. The other technique is mini CPB or miniaturized extracorporeal circuit with unproven efficacy in CSA-AKI prevention^[101]. Off-pump coronary artery bypass (OPCAB) is another technique to ameliorate CPB-related complications and aortic manipulations. However, it is interesting that the effectiveness of OPCAB in preventing CSA-AKI is controversial and still one of the most debated topics in cardiac surgery. Although OPCAB has been shown to be superior in many studies^[102-105], the results of recent large trials results have documented that it does not decrease important endpoints, especially the need for RRT^[106,107]. This may place an emphasis on the importance of hemodynamic stability in AKI prevention on account of the fact that during OPCAB, episodes of hypotension are inevitable. Overall, we conclude that at least in patients with lower risk for AKI, OPCAB may not decrease the likelihood of kidney impairment after cardiac surgery.

Hemodynamic

Perioperative hypotension during CPB increases the incidence of CSA-AKI. It is more important to preserve end-organ function and cellular oxygen delivery during CPB with its unique pressure and nonpulsatile flow characteristics. Thus, it is not the absolute hypotension but perfusion pressure that plays a pivotal role in protecting susceptible organs such as the kidney against CSA-AKI. The kidney medulla is more vulnerable since its oxygen delivery is already low^[108,109]. The difference between preoperative and intraoperative blood pressure may be a more important predictor of CSA-AKI compared to absolute hypotension. A study in 2010 showed that when this difference is more than 25 mmHg the incidence of CSA-AKI increases^[110].

Hemodilution

The carrying capacity of oxygen is influenced by hemo-

dilution which is inevitable during CPB. This adds to hemodynamic changes due to nonpulsatile flow and puts the kidney at danger of ischemia^[109]. It has been suggested that hematocrit levels less than 24% increase the risk of CSA-AKI^[111-113]. However, in all probability, preoperative hematocrit plays an important role^[114]. The most important factor is the balance between oxygen delivery and oxygen consumption which is crucial everywhere in the body, not least in the kidney which is more susceptible to ischemia^[124]. Even the probable risk of hypothermia during CPB may be explained by reperfusion ischemia due to rapid rewarming^[115].

That hemodilution has some adverse effects does not mean that blood transfusion is absolutely beneficial in improving renal function. RBC storage more than 14 d has been associated with increased organ injury^[116]. Moreover, the adverse effects of packed cell transfusion when hemoglobin level is not low outweigh its benefits^[117,118].

Evidence-based blood conservation techniques include increasing preoperative blood volume by drugs such as erythropoietin and decreasing postoperative blood loss (tranexamic acid and aminocaproic acid), preserving the patient's own blood by autologous techniques, such as predonation and intraoperative hemodilution, and intraoperative cell salvage^[119].

CPB flow

Pulsatile flow is believed to improve renal function by decreasing peripheral vascular resistance, optimizing microcirculation and decreasing tissue edema^[120,121]. However, the inconsistent results of studies cannot support its routine use for protection against CSA-AKI^[122-124].

RISK MODELS FOR AKI AFTER CARDIAC SURGERY

Identification and categorization of high-risk patients allows optimal decision-making for earlier intervention and better management, along with the identification of the patients who do not respond to conventional treatments. Risk prediction models can also be used as research tools to select high risk patients for performing studies on AKI. Several risk stratification models have been developed by research groups in patients undergoing different surgeries^[11,125].

As discussed before, CSA-AKI has its own characteristics. Although some risk factors for AKI are common in general and cardiac surgery, the risk scores developed in a general surgery population underestimate the risk of AKI in cardiac surgery^[126]. There are several risk prediction models that have been developed in the field of cardiac surgery^[11,14,15,127-130].

Chertow *et al*^[11] developed the first risk score using a large population database in 1997. This algorithm stratified preoperative risk for dialysis based on data from 43 medical centers gathered in the Continuous Improvement in Cardiac Surgery Study. Then three other predictive risk models were developed, all of which aimed at predicting

the need for dialysis as outcome^[14,127,128]. The most validated model with a high level of precision and the best discriminative power is the Cleveland Clinic Score which was published in 2005 by Thaker *et al*^[14] (Candela-Toha *et al*^[126], Di Bella *et al*^[131], Englberger *et al*^[132] and Heise *et al*^[133]).

In 2006, the Society of Thoracic Surgeons Bedside Risk Tool was developed by Mehta *et al*^[128] through the analysis of a multicenter dataset of more than 600 hospitals. Simplified Renal Index was developed by Wijesundera *et al*^[127] from a Toronto cohort in 2007. Validation studies by other researchers indicate that recalibration of every risk score is needed for optimal risk prediction in any center^[134-136]. Other available models are aimed at predicting AKI not requiring dialysis. They have not been externally validated, however, and due to different definitions of AKI it is difficult to generalize them^[16,129,130].

The most important criticism to the available risk models is their lack of prediction for CSA-AKI. There are still different definitions for AKI and there is no guideline to recommend a specific prediction model^[2,48]. As discussed before, we need to add novel biomarkers to the current risk models and AKI definitions so as to be able to develop scoring systems for the prediction of the earlier stages of AKI. A study by Parikh *et al*^[89] indicated that adding urine IL-18 and plasma NGAL to the risk models improved risk prediction by 25% and 18% respectively.

CREATININE AND THE OUTCOME PREDICTION IN CARDIAC SURGERY

The critical role of creatinine as a strong predictor has been incorporated in the different mortality risk scores that are currently in use for cardiac surgery patients^[3,49,137]. Known risk models have employed a wide range of risk factors from only three to dozens^[3,4]. However, high level of serum creatinine or its equivalents (past history of kidney dysfunction, need for renal replacement therapy and/or dialysis) has been the constituent in almost all of them. The first scoring system was developed by Parsonnet *et al*^[138] in 1989, which included serum creatinine in 14 independent variables. Subsequently, Higgins *et al*^[139] proposed the Cleveland Clinic score in 1992. Cleveland Clinic score was basically developed for coronary artery bypass graft (CABG) operations with or without associated valve surgery and included creatinine among 9 independent variables included in this score. Another mortality predictor introduced in 1992 was the Northern New England score which was developed for isolated CABG operations and included preoperative dialysis dependency as a surrogate for high serum level of creatinine^[140]. Nilsson *et al*^[3] and Magovern *et al*^[141] developed another risk algorithm to be applied in isolated CABG with promising results compared to a group of 18 risk models.

In the last decade, the additive^[142] and the logistic^[143] EuroSCORE predicting tools were developed and subsequently widely validated. These scores are prepared to be applied in all cardiac surgeries in adult patients and

each of them includes 17 independent variables. Serum creatinine receives a score of two when its absolute value is more than 200 $\mu\text{mol/L}$ (2.25 mg/dL). EuroSCORE overestimates mortality and its performance in high risk patients is not good. EuroSCORE II was released in 2012 to improve the accuracy of this measure^[144].

The risk score developed by the Society of Thoracic Surgeons is more complex, built on a database with more than five million records and including several hundred variables^[145]. The risk calculator is available freely at: <http://riskcalc.sts.org/STSWebRiskCalc273/>. On the other hand, Ranucci *et al*^[4] recently proposed a simple score with only three variables, including creatinine, and demonstrated that this risk model was superior to or as effective as the other more complex risk scoring systems. Creatinine in this score has an absolute cut off point of 2 mg/dL. In patients with serum level of creatinine higher than 2 mg/dL, one point is added to sum of the patient's risk. The formula is as follows: age (years)/ejection fraction (%) + 1 (if serum creatinine > 2 mg/dL).

The main weakness of existing risk models is their inaccuracy in different time periods and patients' conditions and various regional settings which emphasizes the dynamic trends in cardiac surgery^[146,147]. Moreover, known risk models are principally prepared to predict mortality. So, we probably are unable to accurately predict morbidity and cost of care by the available risk models. The other major flaw is the lack of a consensus definition of time span for mortality by these models^[137].

PREVENTION AND TREATMENT ON THE HORIZON

Briefly, no treatment or prophylactic measure studied thus far has received sufficient evidence-based support to be employed in AKI management^[66]. However, avoidance of AKI by preventive measures remains the mainstay of management in high risk patients. Contrast induced AKI is probably an exception in that it is preventable and manageable by hydration, N-acetyl cysteine and bicarbonate^[148].

Renoprotective measures include preventive simple maneuvers such as avoidance of nephrotoxic drugs, hydration, glycemic control, maintenance of renal perfusion and goal directed therapy (GDT), as well as more advanced pharmacological interventions^[1,2,70,71] (Table 3).

Preventive measures

Renal injury can be mitigated by two approaches: preventing CSA-AKI from being superimposed on CKD by appropriate risk assessment and preventing subclinical or silent AKI from occurring before cardiac surgery. Management of the adverse effects of contrast dye is an example of the second approach. The role of contrast dye in the occurrence of CSA-AKI is well known and as it is not avoidable, the minimum possible dose of preferably newer non-ionic contrast with lower osmolality should be used^[149,150]. Timing of surgery following contrast angiography may play a role in CSA-AKI. It has been shown

Table 3 Potential preventive measures and pharmacological interventions in acute kidney injury

Preventive measures
Avoidance of nephrotoxic drugs
Angiotensin-converting enzyme inhibitors
Angiotensin receptor blockers
Hydration
Glycemic control
Maintenance of renal perfusion
Goal directed therapy
Ischemic preconditioning
Prevention of CI-AKI
Hydration
N-acetyl cysteine
Bicarbonate
Timing of surgery
Pharmacological interventions
Fenoldopam
Nesiritide
Sodium bicarbonate
Mannitol
Atrial natriuretic peptide
Brain-type natriuretic peptide
Early postoperative renal replacement therapy
Continuous renal replacement therapy
Ultrafiltration

CI-AKI: Contrast induced acute kidney injury.

that cardiac surgery within 24 h of angiography is not safe. In the case of large contrast dose administration it is better to postpone surgery for five days^[151,152].

Sufficient hydration is protective not only in patients at risk of contrast-induced nephropathy^[153], but also in patients with underlying renal insufficiency^[154]. Ideal fluids have been thoroughly investigated to be recognized, without consistent results^[1,71]. It appears from studies to date that colloids are not superior to crystalloids in improving outcome^[155,156]. Moreover, recent studies have shown that contrary to previous belief, clinical application of semisynthetic colloids, especially hydroxyethyl starch solutions, are increasingly difficult to justify in the perioperative period^[156]. Of more importance is probably maintaining normal renal perfusion as 80% of patients diagnosed with AKI after surgery have had an episode of perioperative hemodynamic instability^[157]. GDT, involving the use of enough fluid and blood along with inotropes to optimize hemodynamic parameters and oxygen delivery, is a recommended strategy^[156,158,159]. Fluids are required to be prescribed as a drug^[156]. This is possible through the employment of physiological parameters such as the plethysmographic variability index, stroke volume variation and pulse pressure variation in advanced monitoring systems^[159]. Further studies are needed to optimize protocols^[156,159].

Angiotensin-converting enzyme inhibitors and ARB are potential nephrotoxic medications commonly used in cardiac patients. Avoiding them has not been shown to change the incidence of CSA-AKI and the subject is still controversial^[160,161].

Other measures, such as ischemic preconditioning us-

ing three 5 min intervals of ischemia separated by exactly the same times and interval of reperfusion in the thigh, have been shown to reduce the risk of CSA-AKI^[162].

Pharmacological interventions

Finding a pharmacological agent for the management of CSA-AKI has been challenging due to the absence of standard definitions and end-points^[1,2,26,70,163]. Many drugs have been investigated to date to control the serum level of creatinine and renal protection. Fenoldopam, a selective agonist of dopamine-1 receptor, is the only drug that consistently and significantly has reduced the risk of AKI, followed by nesiritide with initial promising results^[1,164,165].

Sodium bicarbonate was found in a known pilot study in 2009 to decrease the risk of AKI by 20%^[166]. However, another large study in 2012 questioned its usefulness^[167]. This is also true for statins. First reports on its usefulness were not supported by the following studies^[168-171].

It has been known since 2001 that low-dose dopamine is not justified for the prevention and treatment of AKI^[172,173]. Furosemide infusion, especially in combination with dopamine, is even detrimental and may increase postoperative creatinine^[174,175]. Mannitol is an osmotic diuretic that has been used routinely in priming solution for decades. Currently there is debate on its usefulness in cardiac surgery and studies have been inconclusive. However, it is probably reasonable to continue its use as a harmless fluid until strong evidence, guidelines and recommendations are published^[176,177].

Atrial natriuretic peptide and brain-type natriuretic peptide (BNP) are endogenous diuretics with promising effects on renal function in cardiac surgery^[178-180]. BNP is highly associated with postoperative AKI such that it has been considered as a biomarker for AKI in the recent report of TRIBE-AKI study^[181]. Nesiritide, the recombinant human BNP, has been shown to be beneficial according to initial results. Further studies are required before applying nesiritide routinely in daily clinical practice^[182,183].

N-Acetylcysteine has protective effects on contrast-induced nephropathy^[184]. Be that as it may, its prophylactic administration in cardiac surgery is under question. Recent meta-analyses have concluded that current data do not support its routine use in cardiac surgery and it has obtained least strength evidence among prophylactic measures for renal protection^[1,163,185].

Current data is insufficient to support preoperative prophylactic RRT. The best starting time for postoperative RRT is also controversial. Most studies have found lower mortality with the earlier initiation of RRT^[163,186,187]. In addition, recent guidelines suggest that using continuous RRT is superior to standard intermittent RRT in hemodynamically unstable patients^[148]. It is clinically indicated and applicable, although reviews to date have not found differences in survival between the two modes^[188]. Similarly, the benefits of ultrafiltration on CSA-AKI prevalence and severity in adult cardiac surgery warrants further investigation.

CONCLUSION

Recent advances in diagnosis and management of CSA-AKI have opened new perspectives for scientists and medical practitioners. However, creatinine still plays the main role in diagnosis and prediction. New consensus classification for AKI (KDIGO) and new formula for eGFR calculation (CKD-EPI) are promising for better evaluation of patients at risk of postoperative AKI. Incorporating a panel of novel biomarkers in diagnosis and prevention could enhance the quality of the prediction and cause supportive care to be employed earlier. Results of large studies are expected to qualify the capability of these achievements to improve patients' daily care. With respect to the AKI prevention and management, notwithstanding the large number of studies, more attempts are required to reach the optimal prophylactic and therapeutic goals.

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Newer methods of cardiac output monitoring

Yatin Mehta, Dheeraj Arora

Yatin Mehta, Dheeraj Arora, Institute of Critical Care and Anesthesiology, Medanta The Medicity, Haryana 122001, India
Author contributions: Both authors mutually contributed to this paper.

Correspondence to: Yatin Mehta, MD, MNAMS, FRCA, Chairman, Institute of Critical Care and Anesthesiology, Medanta The Medicity, Sector 38, Gurgaon, Haryana 122001, India. yatinmehta@hotmail.com
Telephone: +91-124-4141414

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have lesser complications as compared to gold standard methods.

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Abstract

Cardiac output (CO) is the volume of blood ejected by each ventricle per minute and is the product of stroke volume and heart rate. CO can thus be manipulated by alteration in heart rate or rhythm, preload, contractility and afterload. Moreover it gives important information about tissue perfusion and oxygen delivery. CO can be measured by various methods and thermodilution method using pulmonary artery catheter (PAC) is till date considered as gold standard method. Complications associated with PAC led to development of newer methods which are minimally or non-invasive. Newer methods fulfil other properties like continuous and reproducible reading, cost effective, reliable during various physiological states and have fast response time. These methods are validated against the gold standard with good level agreement. In this review we have discussed various newer methods of CO monitoring and their effectiveness in clinical use.

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Key words: Cardiac output; Pulse contour analysis; Pulse power analysis; Bioimpedance; Doppler; Echocardiography

Core tip: This is review of newer methods of cardiac output monitoring which are minimally invasive and

INTRODUCTION

Cardiac output (CO) monitoring is an important tool in high risk critically ill surgical patients in whom large fluid shifts are expected along with bleeding and hemodynamic instability. It is an important component of goal directed therapy (GDT), *i.e.*, when a monitor is used in conjunction with administration of fluids and vasopressors to achieve set therapeutic endpoints thereby improving patient care and outcome. CO cannot be measured reliably by clinical examination and routine assessment. There are various methods of CO monitoring based on Ficks principle, thermodilution, Doppler, pulse contour analysis and bioimpedance. Each method has its own merits and demerits (Table 1). An ideal CO monitor should be minimally or non-invasive, continuous, cost effective, reproducible, reliable during various physiological states and have fast response time^[1]. Advances in the computer software and hardware have led to development of newer methods of CO monitoring with minimal or no vascular access.

Methods of CO monitoring are broadly classified as follows: (1) Invasive-Intermittent bolus pulmonary artery thermodilution, Continuous pulmonary artery thermodilution; (2) Minimally invasive-Lithium dilution CO (LiD-CO), Pulse contour analysis CO (PiCCO and FloTrac), Esophageal Doppler (ED), transesophageal echocardiography (TEE); and (3) Non-invasive-Partial gas rebreathing, Thoracic bioimpedance and bioreactance, endotracheal cardiac output monitor (ECOM), Doppler method and Photoelectric plethysmography.

Table 1 Advantages and disadvantages of methods of cardiac output monitoring

No	Device	Type	Advantages	Disadvantages
1	PAC	Invasive	Gold standard	Catheter related complications
2	Continuous CO by PAC	Invasive	Continuous CO measurement	Catheter related complications
3	LiDCO	Minimally invasive	Only one arterial line	Cost
			Continuous CO measurements	Requires good arterial waveform
			Measure SV and SVV	Requires Calibration
4	PiCCO	Minimally invasive	Continuous CO measurement	Contraindicated in Lithium therapy
			Effective during hemodynamic instability	Requires good arterial waveform
5	FloTrac	Minimally invasive	Continuous CO measurement	Requires calibration
			No calibration	Requires good arterial waveform
6	PRAM	Minimally invasive	No calibration	Still not validated
7	ED	Minimally invasive	Simple to use	Measure flow only in descending thoracic aorta
			Reliable	Assumptions about aortic size may not be accurate
			Useful in GDT	
8	TEE	Minimally invasive	Evaluate cardiac anatomy preload and ventricular function	Cost
			Ease of use	Skilled personnel
9	Partial non-rebreathing systems	Non invasive	Continuous CO measurement	Affected by changes in dead space or V/Q matching
10	Thoracic bioimpedance	Non invasive	Continuous CO measurement	Affected by electrical noise, movement, temperature and humidity
				Requires hemodynamic stability
				Not useful in dysrhythmias
11	ECOM	Non invasive	Continuous CO measurement	Coronary blood flow not recorded
				Electrocautery produces interference

CO: Cardiac output; LiDCO: Lithium dilution CO; PiCCO and FloTrac: Pulse contour analysis; PRAM: Pressure recording analytic method; ED: Esophageal Doppler; TEE: Transesophageal echocardiography; ECOM: Endotracheal cardiac output monitor; PAC: Pulmonary artery catheter; SV: Stroke volume; SVV: SV variation; GDT: Goal directed therapy.

INVASIVE METHODS

Cardiac output measurement by pulmonary artery catheter

Pulmonary artery catheter (PAC) as a monitor to measure flow and pressure was developed by Dexter^[2] and modified later on by Swan *et al*^[3] to measure CO and central filling pressures. It is still considered as gold standard monitor to measure CO since 1970's^[4]. It has been used as a monitoring tool in high risk surgeries and critical care units.

However, its use has been associated with various complications like pneumothorax, arrhythmia, infection, pulmonary artery rupture, valve injury, knotting and thrombosis leading to embolism^[5,6]. Also, various technical errors may lead to false readings like loss of injectate, variability of temperature, thermistor malfunction, clot over catheter tip, coiling of catheter or timing of injectate > 4 s. Moreover, intracardiac shunts, mechanical ventilation or valvular dysfunction may lead to incorrect readings. These errors and adverse effects led to the development of less invasive methods of CO monitoring^[7,8]. Thus the main objective of present review article is to focus on the newer methods of CO monitoring that are validated with the gold standard method and have ease of use and lesser complications.

CONTINUOUS CO MEASUREMENT BY PAC

Continuous CO (CCO, Edwards Lifesciences, Irvine,

California, United States) is a modification of PAC with copper filament in the catheter that remains in the right ventricle. There is intermittent heating of blood in the right heart by the filament and the resultant signal is captured by thermistor near the tip of the catheter. Average value of CO measured over time is displayed on the monitor. Main advantages of CCO over conventional PAC are avoidance of repeated boluses thus reducing the infection risk and operator errors^[5]. Moreover, continuous monitoring of stroke volume (SV), systemic vascular resistance (SVR) and mixed venous saturation can also be performed with this catheter. We found CCO to be comparable to conventional intermittent thermodilution CO in patients undergoing off pump coronary artery bypass grafting surgery (OPCAB) at various time points^[9].

Literature review regarding use of PAC in operating room and intensive care units (ICU) revealed both benefits and risks. Gore *et al*^[10] showed that PAC use increased mortality after myocardial infarction and SUPPORT trial also showed increased mortality at 30 d^[11]. Complications have led some authors to call for complete moratorium on PAC use^[12]. Various randomized controlled trials (RCT) also demonstrated increased incidence of adverse events in comparison to central venous pressure 1.5% *vs* 0.7% with no significant difference in mortality and length of stay in hospital^[13]. Later on PAC-MAN trial failed to show any benefit or harm with the use of PAC^[14]. Its use in patients undergoing OPCAB also showed no difference in mortality and final outcome^[15]. ESCAPE trial demonstrated functional improvement with PAC guided

therapy used in patients with congestive heart failure^[16].

In spite of various arguments PAC is still considered as the “Gold Standard” for monitoring of CO. However, due to inherent risk associated with its use investigators are trying to develop a minimally or non-invasive monitor for CO which has all the characteristics of an ideal monitor. Various methods based on arterial pulse contour analysis, plethysmography, Fick’s principle or bioimpedance have been developed. Its values should be within limits of agreement (Bland Altman analysis)^[17] of the “gold standard”. We will discuss these methods in the present review.

MINIMALLY INVASIVE METHODS

Pulse power analysis

This method is based on the principle that change of the blood pressure about the mean is directly related to the SV. Various factors affect its accuracy like compliance of the arterial tree, wave reflection, damping of the transducer and aortic systolic outflow^[18].

LiDCO (Cambridge, United Kingdom) system combines pulse contour analysis with lithium indicator dilution for continuous monitoring of SV and SV variation (SVV). Root mean square method is applied to the arterial pressure signal and called “nominal SV” and using a patient specific calibration factor is further scaled to an “actual SV”^[19]. It is a minimally invasive technique first described in 1993^[18] and requires a venous (central or peripheral) line and an arterial catheter. A bolus of lithium chloride is injected into venous line and arterial concentration is measured by withdrawing blood across disposable lithium sensitive sensor containing an ionophore selectively permeable to Li. CO is calculated based on Li dose and area according to the concentration time circulation^[20].

It requires calibration every 8 h and during major hemodynamic changes. It is contraindicated in patients on Li therapy and calibration is also affected by neuromuscular blockers as quaternary ammonium residue causes electrode to drift^[20]. Its accuracy is affected by aortic regurgitation, intraaortic balloon pump (IABP), damped arterial line, post-aortic surgery, arrhythmia and intra or extracardiac shunts^[5,20].

This device has been studied in relation with PAC. Linton *et al*^[18] found good correlation with PAC. Good correlation with PAC has also been found in patients undergoing liver transplantation^[21]. Pearse *et al*^[22] studied it for early goal directed therapy and revealed fewer complications and shorter length of hospital stay.

Pulse contour analysis

It is based on the principle that area under the systolic part of the arterial pressure waveform is proportional to the SV^[23]. It was first described by Erlanger and Hooker in 1904 and suggested that CO was proportional to arterial pulse pressure^[24]. In this method the area is measured post diastole to end of ejection phase divided by aortic

impedance that measures SV. It also measures SVV and pulse pressure variation (PPV) which is useful in predicting fluid responsiveness. SVV is the difference between maximum and minimum SV over the respiratory cycle and is caused by changes in preload with alteration in intrathoracic pressure. In addition to that shape of the arterial waveform (dP/dt), arterial compliance, SVR and patient specific calibration factors are also required for calibration^[24]. In 1970’s first algorithm was developed to continuously analyse the pressure waveform from arterial line^[25].

PiCCO system: The PiCCO system (PULSION medical system, Munich, Germany) was the first pulse contour device introduced and was replaced with PiCCO2 in 2007^[26]. It requires both central venous (femoral or internal jugular) and arterial cannulation (femoral/radial). Indicator solution injected *via* central venous cannula and blood temperature changes are detected by a thermistor tip catheter placed in the artery. Thus, it combines pulse contour analysis with the transpulmonary thermodilution CO to determine hemodynamic variables. It requires manual calibration every 8 h and hourly during hemodynamic instability^[27].

In addition, thermodilution curve can be used to measure intrathoracic blood volume (ITBV), global end diastolic volume (GEDV) and extravascular lung water (EVLW). GEDV and ITBV are a measure of cardiac preload and EVLW (interstitial, intracellular or intra alveolar) is a mean to quantify pulmonary edema. It also measures SVV/PPV which is marker of fluid responsiveness^[28].

PiCCO is a relatively invasive method as it requires both arterial and venous cannulation. Its accuracy may be affected by vascular compliance, aortic impedance and peripheral arterial resistance. Moreover, air bubble, clots and inadequate indicator may also affect the accuracy. Valvular regurgitation, aortic aneurysm, significant arrhythmia and rapidly changing temperature may also affect its accuracy^[29].

Various validation studies have found good correlation with PAC during coronary artery bypass grafting^[30]. However, that is not the case in patients undergoing OPCAB^[31]. In non-cardiac and critically ill patients good correlation has been observed^[32]. Significant errors have been reported during hemodynamic instability requiring recalibration^[33].

FloTrac system: FloTrac (Edwards LifeSciences, Irvine, United States) is a pulse contour device introduced in 2005 and is a minimally invasive method as it requires only an arterial line (femoral or radial). The system does not need any external calibration, is operator independent and easy to use. It is based on the principle that there is a linear relationship between the pulse pressure and SV^[19,34].

The algorithm used in this system uses SD of 2000 arterial waveform points which is calculated by arterial pressure waveform sampled each 20 s at 100 Hz. It in-

corporates characteristics of the arterial waveform with patient specific demographics. The SV is estimated by following equation:

$$SV = SD_{AP} \times \mu$$

SD_{AP} = Standard deviation of data points that reflects pulse pressure.

μ = Conversion factor depends on arterial compliance, mean arterial pressure, waveform characteristics.

Vascular compliance is patient's biometric values (sex, age, height and sex)^[35] and waveform characteristics assessed by skewness (degree of asymmetry) and kurtosis (degree of peakedness) of the individual arterial pressure waveform. A change in vascular tone is represented by skewness and kurtosis. The conversion factor μ enables calculation of SV without external calibration. Second generation devices also developed that calibrate every minute leading to improved CO measurement^[36]. A third generation device with Dynamo tone technology that has automatic adjustment for change in the vascular tone has also been made^[37]. Good arterial waveform quality is a prerequisite for accurate reading of CO. Accuracy is affected in patients with significant arrhythmias, IABP or morbid obesity^[38].

Various studies have validated the efficacy of FloTrac with PAC and find good correlation. We have studied FloTrac with PAC in patients undergoing OPCAB and found good agreement. The mean bias and limits of agreement (2 standard deviations) expressed in liters per minute at respective points of measurement were -0.54 ± 1.12 , -0.37 ± 1.0 , -0.42 ± 1.50 , -0.25 ± 1.18 , -0.31 ± 1.28 , 0.41 ± 1.0 , 0.06 ± 1.50 , and 0.09 ± 1.40 ^[39]. However, in patients with low SVR undergoing liver transplantation or septicemia it is not found as accurate as PAC^[40-42]. It is found to be useful in patients undergoing major abdominal surgery who received GDT^[43]. Moreover, the site of the arterial cannulation is also an important determinant of accuracy. In severe vasoconstriction radial artery reading will underestimate the CO while in volume responsive patient volume redistribution to cerebral circulation will also impair the pulse contour analysis through radial artery^[3].

Pressure recording analytic method: Pressure recording analytic method (PRAM)-MostCare (Vytech, Padova, Italy) measures the area under the curve of arterial waveform. Major advantage is that it does not require external calibration and internal calibration is done by morphology of the arterial waveform. PRAM technology analyses whole cardiac cycle and area under the pressure wave (P/t) is determined^[44]. The P/t is divided into diastolic and systolic phase with 2 impedances based on different characteristics. However the accuracy of this method is still not proven.

EV1000/Volume view: A new calibrated pulse wave analysis method (VolumeView™/EV1000™, Edwards Lifesciences, Irvine, CA, United States) has been developed. It is based on pulse pressure analysis, which is calibrated by transpulmonarythermodilution and is currently

under trial. Its comparison with PICCO2 system in critically ill patients found comparable results^[45]. However; very few studies are available for its validation. We have just finished a study on its use for GDT in OPCAB and found it to be very useful.

Esophageal doppler

Esophageal Doppler uses a flexible probe with transducer at the tip. It is of the size of anorogastric tube and can be placed for longer period in intubated patients. At the midthoracic level it measures flow as it is presumed to be parallel to the descending aorta. Since aorta is considered as a cylinder, the flow can be measured by multiplying cross-sectional area (CSA) and velocity. Doppler ultrasound is used to measure the SV. Once an optimal flow profile has been obtained, the blood flow velocity is determined from the shift in frequency of red blood cells. This is done by the ultrasound processor using the Doppler equation:

$$V = f_d \times c / 2 \times f_0 \times \cos\theta$$

V = velocity of blood, f_d = Doppler shift in frequency, c = speed of ultrasound in tissue (1540 m/s), f_0 = initial ultrasound frequency, and θ = the angle of ultrasound beam in relation to the blood flow.

The velocity-time integral (VTI) is calculated from the area under the velocity-time curve and used as the stroke distance. The area can be calculated by nomogram or direct measurement. Thus SV is calculated as $CSA \times VTI$ and CO is calculated as $SV \times HR$ ^[24]. FTc i.e., corrected time flow can also be determined which is used as measure of cardiac preload^[46].

Major limiting factor is that it measures flow only in descending thoracic aorta which is 70% of total flow. A correction factor needs to be added to compensate aortic arch flow. Moreover discrepancies in flow may be seen in aortic coarctation, aneurysm or crossclamp, IABP and various metabolic states. Various factors like changes in pulse pressure, vascular compliance, volume status or inotropes may affect the CSA. In circulatory failure, it has been shown that CSA should be measured directly to prevent any inaccuracy in readings. Unchanged CSA may lead to underestimation of CO^[24]. Accurate velocity can only be determined by proper positioning of the probe which must be within 20° of the axial flow.

Various studies have compared ED with PAC and found good agreement with low bias. A meta analysis revealed it as a reliable method with low bias with limited efficacy^[47]. ED has also been used in GDT and shown greater improvement in SV and CO with faster recovery and shorter length of stay^[48]. In cardiac surgery, decreased hospital and ICU stay with decreased incidence of gut mucosal perfusion, without major complications has been shown with ED^[49]. We also studied this device in patients undergoing OPCAB and found that in comparison with PAC it cannot be used as a sole method for CO monitoring^[50].

TEE

TEE has now been a widely used monitor in periopera-

tive setting. It is an important tool for the assessment of cardiac structures, filling status and cardiac contractility^[51]. Moreover, aortic pathology can also be detected by TEE. Doppler technique is used to measure CO by Simpson's rule measuring SV multiplied by HR. Flow is measured by area under the Doppler velocity waveform that gives VTI and CSA is calculated by planimetry. Measurement can be done at the level of pulmonary artery, mitral or aortic valve. TEE views used for measurement are mid-esophageal aortic long axis view and deep transgastric long axis view with pulsed and continuous wave Doppler respectively. The ultrasound beam is parallel to the blood flow in transgastric view.

TEE has been validated with PAC with good limits of agreement^[52]. It is a useful tool in hemodynamically unstable patient under mechanical ventilation^[53]. However, a skilled operator is required, limited availability and cost factor are major limitations for its use. Standard TEE probe cannot be kept in the patient for too long. Hemodynamic TEE is a disposable thinner TEE probe which can be left *in situ* for several days.

NON INVASIVE METHODS

Partial gas rebreathing

It is also known as the NICO system (Novamatrix Medical Systems, Wallingford, Conn, United States) or partial gas re-breathing monitor and uses indirect Fick's principle to calculate CO. It is used in intubated patients under mechanical ventilation. At steady state, the amount of CO₂ entering the lungs *via* the pulmonary artery is proportional to the CO and equals the amount exiting the lungs *via* expiration and pulmonary veins.

During 30 s of re-breathing, the amount entering does not change, but the amount eliminated by expiration decreases and endtidal CO₂ increases in proportion to the CO^[24]. CO is calculated according to following formula:

$$CO = VCO_2 / C_vCO_2 - CaCO_2$$

Here VCO₂ is CO₂ consumption, CaCO₂ and C_vCO₂ is arterial and venous CO₂ content respectively. The diffusion rate of carbon dioxide is 22 times more rapid than that of oxygen, it is assumed that no difference in venous CO₂ (C_vCO₂) will occur, whether under normal or rebreathing conditions. A disposable circuit is connected to the ventilator circuit along with infrared CO₂ sensor, pneumotachometer and a rebreathing valve. Partial rebreathing is initiated every three minutes by opening the valve and pulmonary blood flow is calculated by difference between normal and rebreathing ratio^[54].

Major limitation is that tracheal intubation with fixed ventilator setting is required. It is also not very accurate in patients with severe chest trauma, significant intrapulmonary shunt, high CO states and low minute ventilation^[24]. Validation studies have not found accuracy of this device with PAC. Studies have shown underestimation preoperatively and overestimation postoperatively after cardiac surgery^[55]. Thus it has limited clinical applicability in comparison to PAC.

Thoracic bioimpedance

Thoracic bioimpedance (TEB) is a non-invasive method of CO monitoring. Initially it was used by astronauts in 1960s^[56]. It is based on the hypothesis by considering thorax as a cylinder perfused with fluid with specific resistivity. It measures the electrical resistance of the thorax to a high frequency, low amplitude current^[24].

Electrodes six in number are placed (two on either side of neck and four in lower thorax) on the patient and the resistance to current flowing from the outermost to innermost electrodes is measured. The bioimpedance is indirectly proportional to the content of thoracic fluid. Tissue fluid volume, pulmonary and venous blood, and the aortic blood volume all contribute to the TEB measurement. Changes in CO will change the amount of aortic blood and will be reflected in a change TEB^[5]. SV is calculated using the formula^[24]:

$$SV = VEPT \times VET \times EPCI$$

VEPT = volume of electrically participating tissue (gender, height, and weight).

VET = ventricular ejection time taken from the R-R interval.

EPCI = ejection phase contractility index which is indirectly proportional to TEB.

Major limitations like interference with electrocautery, proper electrode placement, patient's movements and arrhythmia may affect its accuracy. Studies in cardiac surgical patients revealed good correlation intraoperatively with a mean bias of -0.28 L/min. Presence of sternal wires, or arrhythmia may lead to inaccurate readings in the postoperative period^[57]. Results were also not encouraging in critically ill patients. Moreover, it has been considered as trend analysis monitor rather than a diagnostic one^[58].

Thoracic bioreactance

Thoracic bioreactance (NICOM device, Cheetah medical, Portland, Oregon) is a modification of TEB which avoids interferences by noise and external sources. It analyses changes in the phase of electrical voltage signal to the current applied across the thorax. Changes in electrical capacitive and inductive properties occurs secondary to change in intrathoracic volume.

The method involves placement of two dual electrodes on either side of the thorax. Sine-wave high-frequency (75 kHz) current is transmitted into the body through one electrode and other electrode is used by the voltage input amplifier. The mean of two will give final value^[59].

Electrocautery also affects its accuracy however if the device receives signal for atleast 20 s over a minute the CO value can be determined. Major advantage is the ease of use in intubated patients, arrhythmias, emergency room (ER), ICU and operating room (OR). Validating studies with PAC showed good correlation between the two methods with minimal bias^[57]. Moreover comparison with pulse contour devices like PiCCO and ED also showed comparable results^[58,60].

ECOM

ECOM (Con-Med, Irvine, Calif, United States) measures CO using impedance plethysmography. It is based on the principle of bioimpedance and current is passed through electrodes attached to endotracheal tube shaft and cuff. Current is passed from electrode on the shaft of endotracheal tube (ETT) and change in impedance secondary to aortic blood flow is detected by electrode on the cuff of ETT. An algorithm calculates SV based on impedance changes and CO can be calculated. Impedance is affected by aortic blood flow^[61].

Electrocautery affects its accuracy and coronary blood flow is not calculated. Moreover the technology is still adequately not validated in humans, is costly and has not become very popular.

Portable doppler device

Ultrasonic Cardiac Output Monitors (USCOM, Sydney, Australia) is a portable device which is non-invasive and uses a probe placed suprasternally to measure flow through the aorta or on the left chest to measure transpulmonary flow^[62]. It uses the Doppler principle as used with ED and TEE. Main advantage is the portability of the device and it can be used with ease in ER, OR, ICU and even in wards. Since it is a non-invasive device it can be used by trained nursing staff and is an important screening tool for postoperative cardiac surgical patients as well.

Major limitations are probe positioning as misalignment of ultrasound beam with blood flow may lead to errors and estimation of proper CSA in various physiological states is also important^[24].

We have used USCOM device in post cardiac surgical patients for both left and right sided CO, CI and SV measurements and found good agreement with PAC. On comparing the right-sided CO, SV, and CI with those of PAC, the mean bias was 0.03 L/min, 1.6 mL, and 0.02 L/min per square meters, respectively. The comparison of left-sided CO, SV, and CI with those of thermodilution revealed a means bias of 0.14 L/min, 1.0 mL, and 0.08 L/min per square meters, respectively^[63]. We further studied this device in OPCAB and found good correlation with PAC. The CO had a mean bias of -0.13 L/min and limits of agreement (mean bias \pm 2SD) at -0.86 and 0.59 L/min^[64].

Photoelectric plethysmography

The Nexfin HD (BMEYE B.V, Amsterdam, Netherlands) is a completely non-invasive pulse pressure analysis device that assesses pulse pressure using photoelectric plethysmography in combination with a volume-clamp technique (inflatable finger cuff). CO is derived by Modelflow method. There are very few validation studies to state its efficacy^[65].

CONCLUSION

There are various newer devices for CO monitoring available in clinical practice that are validated against the

gold standard method. Newer devices have the advantage of being minimally or non-invasive and portable. Hence, a few of them can be used outside the OR and ICU. Validation with PAC and other limitations may still be an obstacle for their use in different clinical scenarios. The criteria for selection of newer devices should be based on the institutional protocol and clinical condition of the patients. More RCT's are needed to prove their efficacy and cost benefit. PAC will remain a gold standard for CO monitoring, however, use of newer devices based on pulse contour analysis, pulse pressure analysis and Doppler methods should be encouraged.

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Does manual thrombus aspiration help optimize stent implantation in ST-segment elevation myocardial infarction?

Diego Fernández-Rodríguez, Luis Alvarez-Contreras, Victoria Martín-Yuste, Salvatore Brugaletta, Ignacio Ferreira, Marta De Antonio, Montserrat Cardona, Vicens Martí, Juan García-Picart, Manel Sabaté

Diego Fernández-Rodríguez, Luis Alvarez-Contreras, Victoria Martín-Yuste, Salvatore Brugaletta, Montserrat Cardona, Manel Sabaté, Department of Cardiology, Hospital Clinic, 08036 Barcelona, Spain

Ignacio Ferreira, Department of Cardiology, Hospital Vall d'Hebrón, 08035 Barcelona, Spain

Marta De Antonio, Department of Cardiology, Hospital Germans Trias i Pujol, 08740 Bdalona, Spain

Vicens Martí, Juan García-Picart, Department of Cardiology, Hospital de la Santa Creu i Sant Pau, 08026 Barcelona, Spain

Author contributions: Fernández-Rodríguez D and Alvarez-Contreras L equally contributed to this work; all the authors contributed to this paper.

Correspondence to: Victoria Martín-Yuste, MD, PhD, Department of Cardiology, Hospital Clinic, Villarroel 170, 08036 Barcelona, Spain. vmartiny@clinic.ub.es

Telephone: +34-93-2275519 Fax: +34-93-2275751

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treated with TA had less prevalence of multivessel disease (39.7% *vs* 54.7%, $P = 0.003$) and higher prevalence of initial thrombolysis in myocardial infarction flow < 3 ($P < 0.001$) than NTA group. There was a higher rate of direct stenting (58.7% *vs* 45.5%, $P = 0.009$), with shorter (24.1 ± 11.8 mm *vs* 26.9 ± 15.7 mm, $P = 0.038$) and larger stents (3.17 ± 0.43 mm *vs* 2.93 ± 0.44 mm, $P < 0.001$) in the TA group as compared to NTA group. The number of implanted stents (1.3 ± 0.67 *vs* 1.5 ± 0.84 , $P = 0.009$) was also lower in TA group.

CONCLUSION: In an "all-comers" STEMI population, the use of TA resulted in more efficient procedure leading to the implantation of less number of stents per lesion of shorter lengths and larger sizes.

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Key words: ST-segment elevation myocardial infarction; Primary percutaneous coronary intervention; Manual thrombus aspiration; Stent; Thrombolysis in myocardial infarction flow

Abstract

AIM: To evaluate the impact of thrombus aspiration (TA) on procedural outcomes in a real-world ST-segment elevation myocardial infarction (STEMI) registry.

METHODS: From May 2006 to August 2008, 542 consecutive STEMI patients referred for primary or rescue percutaneous coronary intervention were enrolled and the angiographic results and stent implantation characteristics were compared according to the performance of manual TA.

RESULTS: A total of 456 patients were analyzable and categorized in TA group (156 patients; 34.2%) and non-TA (NTA) group (300 patients; 65.8%). Patients

Core tip: Thrombus embolization is highly detected in ST-segment elevation myocardial infarction (STEMI) leading to unfavorable clinical outcomes. To prevent thrombus embolization, manual thrombus aspiration (TA) receives a high recommendation during primary percutaneous coronary intervention (PCI) by clinical practice guidelines. However, the TASTE trial, recently published, showing no impact of manual TA on short-term mortality, has reopened the debate about the role of this technique in STEMI. This study is one the first showing that manual TA optimizes stent implantation during primary PCI resulted in more efficient procedures, leading to the implantation of fewer, shorter and larger stents.

Fernández-Rodríguez D, Alvarez-Contreras L, Martín-Yuste V, Brugaletta S, Ferreira I, De Antonio M, Cardona M, Martí V, García-Picart J, Sabaté M. Does manual thrombus aspiration help optimize stent implantation in ST-segment elevation myocardial infarction? *World J Cardiol* 2014; 6(9): 1030-1037 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i9/1030.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i9.1030>

INTRODUCTION

ST-segment elevation myocardial infarction (STEMI) occurs as a result of atherosclerotic plaque rupture or erosion and platelet and coagulation activation leading to thrombus formation and complete coronary occlusion^[1]. Primary percutaneous coronary intervention (PCI) with stent implantation is the preferred method to restore epicardial flow in STEMI^[2,3]. Several thrombectomy devices have been developed with the aim to avoid any suboptimal myocardial reperfusion related to thrombus embolization, which might lead to unfavorable clinical outcome^[4].

The randomized clinical trial (RCT) TAPAS, in particular, showed that manual thrombus aspiration (TA) improved myocardial reperfusion and reduced mortality in STEMI patients at 1-year follow-up^[5,6]. These results, confirmed by other studies^[7-10], including a meta-analysis^[11] of 11321 patients from 20 RCT showing lower rates of late mortality, reinfarction and stent thrombosis in patients underwent manual TA compared with conventional primary PCI, led to a recommendation class IIa for manual TA in patients undergoing primary PCI for STEMI^[12]. Nevertheless, the use of the thrombectomy devices is still controversial and not routine in STEMI patients, especially because some studies have shown no impact on clinical outcome^[13-20], such as the TASTE trial^[21]. This RCT, recently published, did not show any impact of manual TA on mortality or any of several other clinical outcomes at 30 d. Furthermore, the potential effect of TA on optimization of stent implantation has not been elucidated yet.

Therefore, we sought to investigate the factors which can lead to the use of the manual TA in STEMI and its impact on acute angiographic success and stent implantation characteristics in a real-world STEMI population.

MATERIALS AND METHODS

Study population

Between May 2006 and August 2008, all consecutive patients with STEMI referred to our hospital for primary or rescue PCI were enrolled. There were no exclusion criteria. Clinical and angiographic characteristics of all patients were prospectively collected. All patients signed a written informed consent prior to PCI procedure and agreed to be clinically followed. At the time of the study an IRB approval was not formally necessary for observational registries that use a CE-mark approved device.

Procedure

Patients treated with primary PCI were pretreated with aspirin (300 mg), clopidogrel loading dose (300 mg) and unfractionated heparin adjusted to weight. The use of glycoprotein (GP) IIb/IIIa inhibitors was left at the discretion of the operators in case of significant thrombus, slow or non-reflow of thrombotic complications. PCI was performed according to conventional clinical practice. Manual TA; using the 6-French Pronto V3[®] aspiration catheter (Vascular Solutions Inc, Minneapolis, MN) and the 6-French Export[®] aspiration catheter (Medtronic, Minneapolis, MN), was performed according to the operator's choice; and patients were thereafter classified in TA group and non-thrombus aspiration (NTA) group.

Manual TA technique was performed as follows. The aspiration was started 2-cm before the culprit lesion and the aspiration catheter was advanced very slowly, crossing the lesion with continuous aspiration. The catheter was removed under aspiration even into the guiding catheter, with generous backflow after retrieving the thrombectomy device. At least two or three passages were performed. Manual TA was especially considered, in case of high thrombus burden and initial slow thrombolysis in myocardial infarction (TIMI) flow.

Definitions and end-points

Time to treatment was defined as time from symptom onset to initial intracoronary therapy by TA or balloon inflation of the infarct-related coronary artery^[22].

TIMI flow grade was evaluated pre guide-wire and post-PCI^[6].

No-reflow was defined as a TIMI flow grade < 2 in absence of coronary dissection, coronary hematoma, occlusive coronary thrombosis or epicardial spasm^[10]. Thrombus embolization was defined as circumscribed filling defects and/or abrupt cut off of a vessel distal to the target lesion or in other coronary vessel on the angiogram after PCI^[23]. Coronary dissection was defined by the presence of a curvilinear filling defect parallel to the vessel lumen, contrast medium outside of the vessel lumen persisting after passage of contrast medium, or a spiral-shaped filling defect partially or totally obstructing the coronary artery lumen^[24].

ST was defined and categorized, according to Academic Research Consortium^[25]. Angiographic success was defined as final TIMI flow equals 3 plus absence of any angiographic complication.

The angiographic assessment was performed by consensus of two independent experienced interventional cardiologists. The primary end-point of this study was the rate of angiographic success, as above defined. Secondary end-points included technical and clinical issues related to the procedure as the number of implanted stents, the rates of direct stenting and post-dilatation, the maximal diameter of the implanted stents, the total stented length segment, the final TIMI flow and the resolution of the ST-segment elevation after primary PCI.

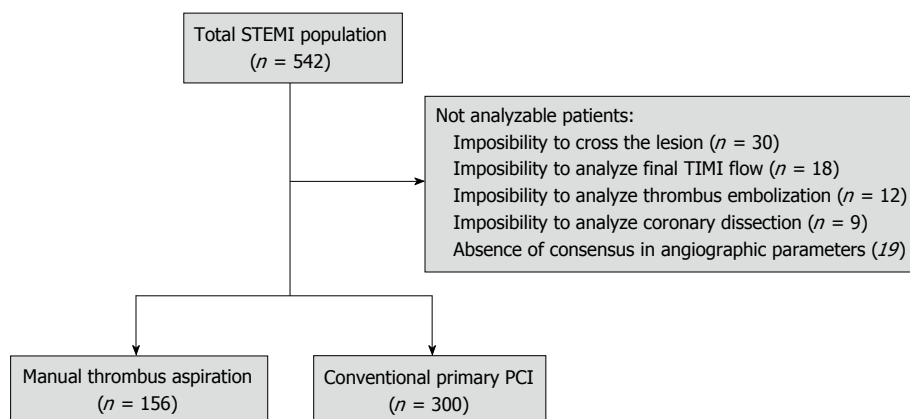


Figure 1 Study flowchart. STEMI: ST-segment elevation myocardial infarction; PCI: Percutaneous coronary intervention; TIMI: Thrombolysis in myocardial infarction.

Clinical follow-up

A clinical follow-up up to 3 years was performed by a clinical visit or telephone interview. Clinical outcomes were evaluated by measuring the rate of the major adverse cardiac events (MACE) defined as the combination of cardiac death, myocardial infarction (MI) and need for cardiac artery by-pass grafting (CABG) and its individual components, as well as the rate of all-cause death, and the need for target vessel and non-target vessel PCI revascularization. MI was defined according to the World Health Organization extended definition^[20].

Statistical analysis

Continuous variables were explored for normal distribution with the Kolmogorov-Smirnov test. Normally distributed variables were expressed as mean (1 standard deviation) and non-normally distributed variables were expressed as median (inter-quartile range) and were compared using *t*-student or with Mann-Whitney tests as appropriate. Categorical variables were expressed as count (percentage) and were compared using the χ^2 test.

In order to exclude confounding factors in primary end-point (angiographic success), multivariable logistic regression models were fitted to assess independent predictors. The following variables were tested for the predictors of the primary end-point: manual TA, age, gender, smoking history, prior MI, primary PCI, Killip class > I, initial TIMI flow = 0, use of GP II b/IIIa inhibitors and the use of drug-eluting stents (DES). The result was reported as HR together with the 95%CI.

All *P* values were 2-tailed, with statistical significance set at a level of < 0.05. Statistical analyses were performed using SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, United States).

RESULTS

Baseline clinical and angiographic features

A total of 542 patients were prospectively included during the recruitment period. Of them, 30 patients were not analyzable because impossibility to crossing the culprit lesion by the TA device and 56 patients because

inability to analyze the angiographic data. The remaining 456 patients were finally studied and classified in TA (*n* = 156) and NTA groups (*n* = 300) (Figure 1).

Baseline characteristics are presented in the Table 1. TA group exhibited lower prevalence of dyslipidemia (19.2% *vs* 30.7%, *P* = 0.009) and multivessel disease (39.8% *vs* 54.7%, *P* = 0.003) in comparison with NTA group. Conversely, TA was more often used in primary PCI (73.1% *vs* 68.7%, *P* = 0.013), in presence of initial TIMI flow < 3 (*P* < 0.001), and with concomitant use of GP II b/IIIa inhibitors (65.3% *vs* 50.6%, *P* = 0.012) in comparison with NTA group.

Procedural results

Main procedural results are presented in the Table 2. Patients included in TA group showed higher prevalence of angiographic success (78.8% *vs* 68%, *P* = 0.015) and better final TIMI flow (TIMI flow 3: 85.9% *vs* 78.3%, *P* = 0.04) in comparison with NTA group. Patients treated with TA received higher rate of direct stenting (58.7% *vs* 45.5%, *P* = 0.009), less number of stents implanted (1.3 ± 0.67 *vs* 1.5 ± 0.84 , *P* = 0.009), with larger (3.17 ± 0.43 mm *vs* 2.93 ± 0.44 mm, *P* < 0.001) and shorter sizes (24.1 ± 11.8 mm *vs* 26.9 ± 15.7 mm, *P* = 0.038). The use of DES was lower in the TA group (DES; 11.3% *vs* 16.3%, *P* = 0.008). In multivariate analysis, TA was associated with angiographic success (HR = 2.3; 95%CI: 1.2-4.3) (Table 3).

In-hospital and long-term outcomes

In-hospital and long-term data are presented in the Table 4. No difference in major cardiac events was observed between groups during hospitalization. The only difference was a significantly higher CK peak [2563 (1284-4542) UI/L *vs* 1517 (744-3816) UI/L, *P* = 0.02] observed by the use of TA.

At three years clinical follow-up (36 ± 7 mo), no differences between manual TA and conventional PCI were observed in the rates of MACE (17.0% *vs* 21.6%, *P* = 0.25), all-cause death (17.0% *vs* 19.6%, *P* = 0.5), cardiac death (8.3% *vs* 7.9%, *P* = 0.83), MI (6.8% *vs* 10%, *P* = 0.27), need for CABG revascularization (1.4% *vs* 3.5%, *P* = 0.39),

Table 1 Baseline clinical and angiographic features *n* (%)

Characteristics	Thrombus aspiration <i>n</i> = 156	Conventional PCI <i>n</i> = 300	<i>P</i> value
Age, mean ± SD	63.2 ± 12.8	64.3 ± 12.8	0.410
Female sex	38 (24.4)	62 (20.7)	0.370
Previous or current smoker	94 (60.3)	205 (68.3)	0.085
Hypertension	82 (52.6)	166 (55.3)	0.570
Dyslipidemia	30 (19.2)	92 (30.7)	0.009
Peripheral vasculopathy	9 (5.8)	19 (6.3)	0.800
Previous MI	10 (6.7)	36 (12.3)	0.065
Previous PCI	8 (5.1)	29 (9.7)	0.092
Previous CABG	2 (1.3)	10 (3.3)	0.190
Indication			0.013
Primary	114 (73.1)	206 (68.7)	
Rescue	42 (26.9)	94 (31.3)	
Classification			0.650
Anterolateral	69 (44.2)	133 (44.3)	
Inferoposterior	83 (53.2)	152 (50.7)	
Non-Q MI	3 (1.9)	12 (4)	
LBBB	1 (0.6)	3 (1)	
Killip			0.058
I	182 (86.3)	228 (76.5)	
II	13 (8.5)	35 (11.7)	
III	1 (0.7)	7 (2.3)	
IV	7 (4.6)	28 (9.4)	
Number of diseased vessels			0.003
1	94 (60.3)	136 (45.3)	
2	43 (27.6)	95 (31.7)	
3	19 (12.2)	69 (23)	
Infarct related artery			0.650
LAD	68 (43.6)	137 (45.7)	
LCx	15 (9.6)	35 (11.7)	
RCA	69 (44.2)	116 (38.7)	
LM	4 (2.6)	8 (2.75)	
Bypass	0	1 (0.3)	
GP II b/IIIa inhibitors			0.012
IABP	7 (4.5)	25 (8.4)	0.120

PCI: Percutaneous coronary intervention; MI: Myocardial infarction; CABG: Coronary artery by-pass graft; LBBB: Left bundle branch block; LAD: Left anterior descending; LCx: Left circumflex; RCA: Right coronary artery; LM: Left-main; IABP: Intra-aortic balloon pump; GP: Glycoprotein.

target vessel PCI revascularization (5.4% *vs* 8.9%, *P* = 0.2), and non-target vessel PCI revascularization (4.8% *vs* 5.7%, *P* = 0.68) and definite ST (1.4% *vs* 4.4%, *P* = 0.15).

DISCUSSION

The major findings of this study were: (1) manual TA was used more often in primary PCI and in patients with worse TIMI flow; (2) its use was subsequently related to optimization of procedural technique; and (3) TA was independently associated with acute angiographic success.

Optimization of angiographic outcomes and stent implantation by manual TA in real-world

According to clinical trials and real-world registries, our work confirms that manual TA is more often used in the

Table 2 Procedural data and angiographic results *n* (%)

Characteristics	Thrombus aspiration <i>n</i> = 156	Conventional PCI <i>n</i> = 300	<i>P</i> value
Time to treatment, median (IQR)	273 (170-477)	300 (180-480)	0.610
Initial TIMI flow			< 0.001
0	111 (71.2)	143 (48.8)	
1	9 (5.8)	16 (5.5)	
2	16 (10.3)	38 (13)	
3	20 (12.8)	96 (32.8)	
Initial TIMI flow < 3	136 (87.2)	204 (67.2)	< 0.001
Final TIMI flow			0.140
0	2 (1.3)	9 (3.1)	
1	1 (0.6)	6 (2.1)	
2	19 (12.3)	50 (17.1)	
3	133 (85.8)	227 (77.7)	
Final TIMI flow < 3	22 (14.1)	65 (21.7)	0.040
Angiographic complication			0.450
Non-reflow	6 (3.8)	16 (5.4)	
Thrombus embolization	7 (4.5)	22 (7.4)	
Coronary dissection	2 (1.3)	7 (2.4)	
Angiographic success	123 (78.8)	200 (68)	0.015
Direct stenting	88 (58.7)	131 (45.5)	0.009
Type of stent			0.008
BMS	133 (88.7)	238 (79.3)	
DES	17 (11.3)	62 (20.7)	
Length of stented segment (mm), mean ± SD	24.1 ± 11.8	26.9 ± 15.7	0.038
Diameter of stented segment (mm), mean ± SD	3.17 ± 0.43	2.93 ± 0.44	< 0.001
Number of stents, mean ± SD	1.3 ± 0.67	1.5 ± 0.84	0.009
LVEF, mean ± SD	49.6 ± 9.8	49 ± 10.4	0.610

PCI: Percutaneous coronary intervention; IQR: Interquartile range; TIMI: Thrombolysis in myocardial infarction; BMS: Bare metal stents; DES: Drug-eluting stents; LVEF: Left ventricle ejection fraction.

presence of high thrombus burden, such as in patients with initial low TIMI flow (0-1) or primary PCI indication. This registry confirms as well that use of TA achieves better angiographic results than conventional PCI, with greater reduction in thrombus burden and higher rate of final TIMI flow 3. Of note is the recent article by Ahn *et al*^[27] which showed that the addition of II b/IIIa inhibitors (Abciximab) to manual TA improves the index of micro-circulatory resistance and the microvascular obstruction assessed by cardiac magnetic resonance. This leads us to hypothesize that the optimal strategy to optimize myocardial perfusion would be the synergistic use of these two therapeutic options.

Moreover, it appeared that the use of TA allowed immediate good angiographic results before stent implantation, so that fewer, larger and shorter stents could be more often implanted. Previous clinical trials and real-world registries failed to show any differences in the length, diameter and number of implanted stents be-

Table 3 Multivariate analysis of angiographic success

	HR (95%CI)	P
Thrombus aspiration	2.3 (1.2-4.3)	0.007
Primary PCI	4.4 (2.1-9)	< 0.001
Active smoking	1.76 (0.9-3.4)	0.093
Age	1.031 (1.001-1.063)	0.044
Initial TIMI flow = 0	0.46 (0.25-0.84)	0.012

PCI: Percutaneous coronary intervention; TIMI: Thrombolysis in Myocardial Infarction.

Table 4 In-hospital and long-term outcomes *n* (%)

	Thrombus aspiration <i>n</i> = 156	Conventional PCI <i>n</i> = 300	P value
In-hospital			
CK peak UI/L, median (IQR)	2563 (1284-4542)	1517 (744-3816)	0.020
ST resolution at 30 min	75 (71.4)	174 (74)	0.610
Intra-procedural death	2 (1.3)	5 (1.7)	1.000
In-hospital cardiac death	15 (9.6)	22 (7.3)	0.400
Non-target vessel PCI revascularization CABG	12 (7.7)	41 (13.8)	0.059
	0 (0)	1 (0.3)	1.000
Follow-up			
MACE	25 (17.0)	61 (21.6)	0.250
All-cause death	26 (17.0)	57 (19.6)	0.500
Cardiac death	13 (8.3)	23 (7.9)	0.830
MI	10 (6.8)	28 (10)	0.270
CABG	2 (1.4)	10 (3.5)	0.390
Target vessel PCI revascularization	8 (5.4)	25 (8.9)	0.200
Non-target vessel PCI revascularization	7 (4.8)	16 (5.7)	0.680
Definitive stent thrombosis	2 (1.4)	12 (4.4)	0.150
Probable stent thrombosis	1 (0.7)	2 (0.7)	1.000
Possible stent thrombosis	2 (1.4)	6 (2.2)	0.720

PCI: Percutaneous coronary intervention; CABG: Coronary artery by-pass graft; MACE: Major adverse cardiac events; MI: Myocardial infarction; IQR: Interquartile range; ST: Stent thrombosis.

tween patients treated with or without TA^[6,7,10,20,28] except for one brief work^[29] that demonstrated a higher stent diameter after manual TA, in STEMI patients treated with bare metal stents. Recently, the TASTE trial^[21] also showed the need for fewer stents per procedure in manual TA group in comparison with conventional PCI. It is well known that intra-stent restenosis and ST are directly related to the characteristics of the stents^[30,31]. Thus; optimizing on stent implantation using fewer stents and stents of larger diameter and smaller length, during STEMI could have long-term prognostic implications by reducing the intra-stent restenosis and ST.

Besides, in light of these results we might hypothesize that TA may be cost-saving. Therefore, further studies on

cost-effectiveness implications by the use of manual TA in primary PCI are warranted.

Clinical outcomes of TA in real-world

This registry reflected real-world clinical practice in STEMI population as no exclusion criteria was applied. Additionally, both primary and rescue PCI patients were included.

Unlike other studies with strict inclusion criteria^[6,10], this registry demonstrated no impact of TA on both short and long-term outcomes. In the TAPAS trial^[5,6], only patients with primary PCI were included; in another real-world registry^[10], only patients with primary PCI indication and TIMI flow 0-1 were included. Conversely, our clinical results are consistent with studies with broad inclusion criteria, such as the TASTE trial^[21], that evaluated the primary end-point at short-term and with the largest published real-world registry in manual TA^[20] that had a very extended follow-up. Both studies included patients with initial TIMI flow from 0 to 3 and rescue or primary PCI indication. Thus, differences in inclusion criteria and in follow-up periods between the various trials and inherent selection bias induced in clinical registries may explain the different impact of the TA on long-term outcome.

Furthermore, it is noteworthy that in our study MACE rate was numerically higher in NTA group, although it did not reach statistical significance, probably due to the small number of patients included in our registry.

Of note is that no difference in target-vessel revascularization or stent thrombosis was found between the two groups, despite implantation of larger and shorter stent in TA than NTA group: this finding may be explained by the higher rate of DES implanted in NTA group than TA group.

This interesting controversy will continue until the publication of the results of the TOTAL trial^[32]. The TOTAL trial is a multicenter, prospective, open, international, randomized trial with blinded assessment of outcomes which will recruit 10700 STEMI patients to compare routine manual TA with the Export aspiration catheter *vs* conventional primary PCI alone. The primary outcome will be the composite of cardiovascular death, recurrent myocardial infarction, cardiogenic shock, or new or worsening New York Heart Association class IV heart failure up to 180 d.

Study limitations

First, this study is a non-randomized, prospective registry and there were differences in baseline clinical and angiographic characteristics that could lead to a worse baseline risk profile in NTA group. Second, the use of GP II b/IIIa inhibitors was higher in the TA group and this difference could also affect angiographic results in this group. Third, in our study manual TA was only used in one third of cases, whereas current use of manual TA in recent all-comer RCT^[33-35] is around two thirds of patients. This was related to the relatively lack of evidence of manual thrombectomy at the time of the recruitment of the registry. Fourth, the relative small number of patients

included in our study could preclude any conclusions regarding clinical efficacy of TA.

In this all-comer registry, TA was able to optimize stent implantation technique, leading to the implantation of less number of stents per lesion of shorter lengths and larger sizes, and was associated with angiographic success following PCI for STEMI.

COMMENTS

Background

In ST-segment elevation myocardial infarction (STEMI) patients, manual thrombus aspiration (TA) is effective to reduce thrombus burden. Nevertheless, the effect on optimization of stent implantation has not been elucidated yet. Therefore, the objective of this study is to evaluate the impact of manual TA on acute angiographic success and stent implantation characteristics in a real-world STEMI.

Research frontiers

Manual TA reduces thrombotic burden, receiving a recommendation class IIa during the performance of primary percutaneous coronary intervention. However, the TASTE trial, recently published, showing no impact of manual TA on 30-d mortality, has reopened the debate about the role of this technique in STEMI setting.

Innovations and breakthroughs

Thrombus embolization is detected up to 15% of STEMI population and is responsible for suboptimal myocardial reperfusion, leading to unfavorable clinical outcomes. Manual TA reduces thrombotic burden and receives a high recommendation during the performance of primary percutaneous coronary intervention. The TASTE trial, demonstrating absence of impact of manual TA on short-term mortality, has reopened the debate about the use of this technique in STEMI patients. In the present study the authors want to investigate, in a real-world STEMI population, the factors which can lead to the use of manual thrombectomy in STEMI and its impact on angiographic and stent implantation characteristics.

Applications

The study results suggest that manual TA during primary percutaneous coronary intervention is associated with a higher rate of angiographic success and optimization on stent implantation compared with conventional primary percutaneous coronary intervention, in a real-world population. However, it seems to have no impact on long-term clinical outcomes.

Terminology

STEMI: It is a type of acute coronary syndromes, which occurs when a coronary artery becomes totally blocked by a blood clot, causing the heart muscle supplied by the artery to die; Primary percutaneous coronary intervention: It is a non-surgical procedure used to open the occluded coronary arteries during STEMI; Manual TA device: It is a type of thrombectomy device, which comprises a monorail catheter with a central lumen connected proximally to a syringe for manual aspiration, designed to extract thrombotic material during percutaneous coronary intervention.

Peer review

In this study, Diego *et al* reported that the thrombus aspiration therapy in patients with AMI were associated with high procedure success and contributed to optimize the implantation of stents. As a non-randomized, prospective registry study, it provide their some new insights about the use of thrombus aspiration in the real world.

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Calcific left atrium: A rare consequence of endocarditis

Giuseppe Dattilo, Carmelo Anfuso, Matteo Casale, Vincenza Giugno, Lorenzo Camarda, Natascia Laganà, Gianluca Di Bella

Giuseppe Dattilo, Matteo Casale, Vincenza Giugno, Lorenzo Camarda, Natascia Laganà, Gianluca Di Bella, Department of Clinical and Experimental Medicine, Section of Cardiology, University of Messina, 98125 Messina, Italy

Carmelo Anfuso, Division of Radiology, 'Ospedali Riuniti Papardo-Piemonte' Hospital, 98125 Messina, Italy

Author contributions: Dattilo G and Di Bella G undertook the patient clinical examination and echocardiogram; Casale M, Giugno V, Camarda L and Laganà N collected the patient's clinical data and wrote the paper; Anfuso C performed CT and CMR; Dattilo G and Di Bella G analyzed the data.

Correspondence to: Giuseppe Dattilo, MD, PhD, Department of Clinical and Experimental Medicine, Section of Cardiology, University of Messina, Policlinico G. Martino, Via Consolare Valeria, 98125 Messina, Italy. giu.dattilo@libero.it

Telephone: +39-90-2213531 Fax: +39-90-2213530

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Abstract

Usually, cardiac calcifications are observed in aortic and mitral valves, atrio-ventricular plane, mitral annulus, coronary arteries, pericardium (usually causing constrictive pericarditis) and cardiac masses. Calcifications of atrial walls are unusual findings that can be identified only using imaging with high spatial resolution, such as cardiac magnetic resonance and computed tomography. We report a case of a 43-year-old patient with no history of heart disease that underwent cardiac evaluation for mild dyspnoea. The echocardiogram showed a calcific aortic valve and a hyper-echogenic lesion located in atrio-ventricular plane. The patient was submitted to cardiac magnetic resonance and to computed tomography imaging to better characterize the localization of mass. The clinical features and location of calcified lesion suggest an infective aetiology causing an endocarditis involving the aortic valve, atrio-ventricular plane and left atrium. Although we haven't data to support a definite and clear diagnosis, the clinical features and location of the calcified lesion suggest

an infective aetiology causing an endocarditis involving the aortic valve, atrio-ventricular plane and left atrium. The patient was followed for 12 mo both clinically and by electrocardiogram and echocardiography without worsening of clinical, electrocardiographic and echocardiographic data. Cardiac magnetic resonance imaging and computed tomography are ideal methods for identifying and following over time patients with calcific degeneration in the heart.

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Key words: Endocarditis complications; Left atrium calcification; Cardiac magnetic resonance; Computed tomography

Core tip: A patient was submitted to echocardiography, cardiac magnetic resonance and to computed tomography imaging to better characterize a hyper-echogenic lesion located in the atrio-ventricular plane. The clinical features and location of the calcified lesion suggest an infective aetiology causing an endocarditis involving the aortic valve, atrio-ventricular plane and left atrium.

Dattilo G, Anfuso C, Casale M, Giugno V, Camarda L, Laganà N, Di Bella G. Calcific left atrium: A rare consequence of endocarditis. *World J Cardiol* 2014; 6(9): 1038-1040 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i9/1038.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i9.1038>

INTRODUCTION

Calcification can be observed in many cardiac localizations but is particularly rare as a lesion that involves the aortic valve, atrioventricular plane and left atrium.

CASE REPORT

We report a case of a 43-year-old patient with no history

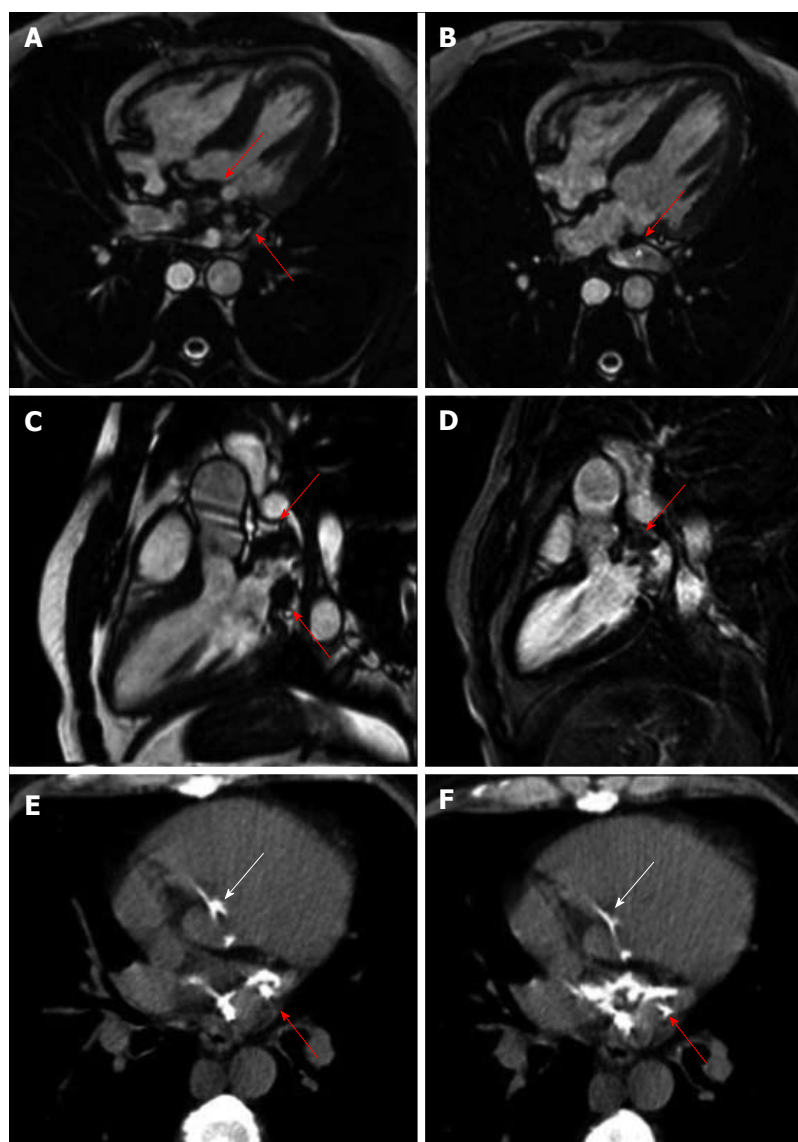


Figure 1 Photograph. A-D: Cardiac magnetic resonance showed hypointense areas located in left atrium and atrio-ventricular plane (red arrows); B: Partial obstruction of superior pulmonary vein; E and F: Cardiac computed tomography showed the presence of a mass suggestive of calcium in left atrium (red arrows), atrioventricular groove and aortic left ventricular outflow (white arrows).

of heart disease who underwent cardiac evaluation for mild dyspnoea. On physical examination he showed only a mild aortic systolic murmur. Blood pressure (130/65 mmHg) and electrocardiogram were normal. The echocardiogram showed an increase of left ventricular (LV) outflow aortic velocity (max velocity 2.2 m/s) due to calcific aortic valve and a hyper-echogenic lesion located in the atrio-ventricular plane. The patient was submitted to cardiac magnetic resonance (CMR) and to computed tomography imaging to better characterize the localization of mass.

CMR by steady-state free precession sequence showed normal atrial and ventricular dimensions; furthermore hypointense areas located in the left atrium and atrio-ventricular plane (Figure 1, red arrows on panel A-D) with a partial obstruction of superior pulmonary vein (Figure 1, on panel B) were found. A gradient echo T1-weighted image after 10 min of injection of contrast media (delayed

contrast enhancement technique) showed a hypointense area in left atrial (LA) suggesting calcium.

Axial images by cardiac computed tomography showed the presence of a mass suggestive of calcium in LA (Figure 1, red arrows on panel E-F), atrioventricular groove and aortic LV outflow (white arrows on panel E-F).

The patient was followed for 12 mo both clinically and by electrocardiogram and echocardiography without worsening of clinical, electrocardiographic and echocardiographic data.

DISCUSSION

Calcification can be observed in many cardiac localizations^[1-7]; particularly, they can be located: (1) valves (usually aortic and mitral valve); (2) atrio-ventricular plane; (3) mitral annulus (usually located in mitral posterior annulus as consequence of a degenerative disorders in the elderly,

osteoporosis women, kidney disease); (4) epicardial coronaries; (5) cardiac masses (caseous calcification of the posterior mitral annulus, soft tissue calcified sarcomas, calcified echinococcus cysts, cardiac osteochondromas and cardiac calcified amorphous tumors); and (6) in pericardium (usually causing constrictive pericarditis).

The calcifications of atrial walls are unusual findings that can be identified only using imaging with high spatial resolution, such as cardiac magnetic resonance and computed tomography. Cardiac magnetic resonance imaging and computed tomography, having a high spatial resolution and tissue characterization, are ideal methods for identifying and following over time patients with unusual localization of calcific degeneration in the heart. This case report represents a very rare manifestation of extended endocarditis. Although we haven't data to support a definite and clear diagnosis, the clinical features and location of the calcified lesion suggest an infective aetiology causing an endocarditis involving the aortic valve, atrio-ventricular plane and left atrium.

COMMENTS

Case characteristics

A 43-year-old patient with no history of heart disease who underwent cardiac evaluation for mild dyspnoea.

Clinical diagnosis

At physical examination there was only a mild aortic systolic murmur.

Imaging diagnosis

Cardiac magnetic resonance (CMR) by steady-state free precession sequence showed hypointense areas located in the left atrium and atrio-ventricular plane with a partial obstruction of the superior pulmonary vein and the delayed contrast enhancement technique showed a hypointense area in left atrial (LA) suggesting the presence of calcium. Axial images by cardiac computed tomography showed the presence of a mass suggestive of calcium in LA, atrioventricular groove and aortic left ventricular outflow.

Related reports

Endocarditis is a serious condition that can endanger patient life, showing itself in different ways.

Term explanation

CMR delayed contrast enhancement technique is based on the use of gradient echo T1-weighted images 10 min after the injection of contrast medium and it

is very useful to evaluate the tissue characteristics, particularly in an organ in constant motion like the heart.

Experiences and lessons

This case report not only represents one of the largest extensions of endocarditis described but also shows a lack of correlation between clinical manifestation and clinical symptoms.

Peer review

The report is interesting, and it is an excellent work.

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Systemic venous atrium stimulation in transvenous pacing after mustard procedure

Calogero Puntrello, Fabiana Lucà, Gaspare Rubino, Carmelo Massimiliano Rao, Sandro Gelsomino

Calogero Puntrello, Fabiana Lucà, Gaspare Rubino, Department of Cardiology, Paolo Borsellino Hospital, 91025 Marsala, Italy

Carmelo Massimiliano Rao, Department of Cardiology, Melacchino Morelli Hospital, 89129 Reggio Calabria, Italy

Sandro Gelsomino, Department of Heart and Vessels, Careggi Hospital, 50100 Florence, Italy

Author contributions: Puntrello C and Lucà F contributed equally to the paper; Puntrello C and Lucà F contributed to concept/design, drafting of the manuscript; Rubino G wrote the paper; Rao CM contributed to critical revision of the manuscript; Gelsomino S contributed to final approval of the manuscript.

Correspondence to: Fabiana Lucà, MD, Department of Cardiology, Paolo Borsellino Hospital, C. da Cardilla, 91025 Marsala, Italy. fabiana.luca@alice.it

Telephone: +39-34-94122107 Fax: +39-55-923753090

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lead usually screwed into the left atrial appendage. The case presented demonstrates that, when the systemic venous atrium is separate from the left atrial appendage, the lead can be easily and safely placed in the systemic venous left atrium gaining satisfactory sensing and pacing thresholds despite consisting partially of pericardial tissue.

Puntrello C, Lucà F, Rubino G, Rao CM, Gelsomino S. Systemic venous atrium stimulation in transvenous pacing after mustard procedure. *World J Cardiol* 2014; 6(9): 1041-1044 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i9/1041.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i9.1041>

Abstract

We present the case of a young woman corrected with a Mustard procedure undergoing successful transvenous double chamber pacemaker implantation with the atrial lead placed in the systemic venous channel. The case presented demonstrates that, when the systemic venous atrium is separate from the left atrial appendage, the lead can be easily and safely placed in the systemic venous left atrium gaining satisfactory sensing and pacing thresholds despite consisting partially of pericardial tissue.

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Key words: Cardiac pacing; Mustard procedure; Transposition of great arteries

Core tip: Disturbances of rhythm in patients undergoing Mustard Procedure are common and they often require implantation of a permanent pacemaker with the atrial

INTRODUCTION

The mustard operation (MO)^[1] was a well-established method to correct the transposition of the great arteries before being superseded, in the recent years, by anatomic repair, the so called arterial switch operation^[2,3]. The procedure employs a pericardial baffle to change the direction of the blood flow from the systemic venous return to the left ventricle and pulmonary venous return to the right ventricle^[1].

Disturbances of rhythm and conduction in patients undergoing MO have been the focus of many studies^[4-6]. Occasionally a permanent pacemaker is needed especially for patients with symptomatic sick sinus syndrome.

Usually one electrode is put in the apex of the anatomic left (subpulmonary) ventricle and the atrial lead is fixed into the left atrial appendage^[4].

Nonetheless, if the systemic venous atrium does not include the left atrial appendage it is impossible to screw the atrial lead into the left atrial appendage. In addition, it is questionable whether, positioning the electrode in the systemic venous atrium, sensing capabilities are inadequate as the neo-atrium consists partially of

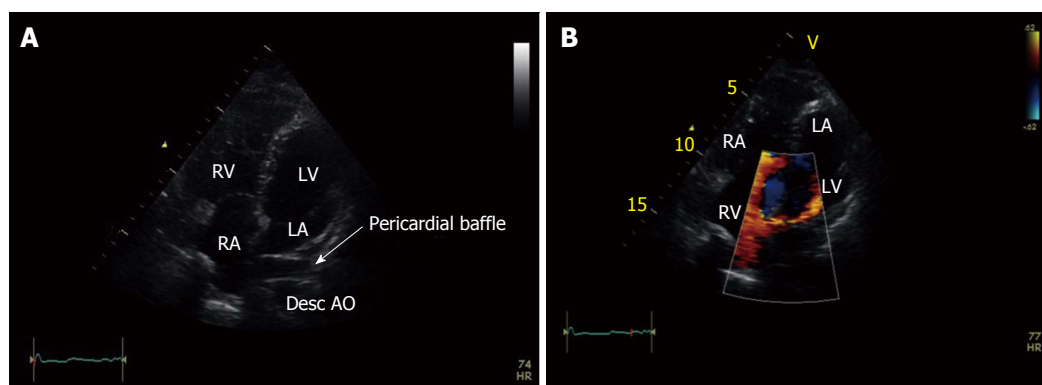


Figure 1 Apical 4-chamber view. A: The Mustard procedure employs a pericardial baffle to direct systemic venous return to the left ventricle and pulmonary venous return to the right ventricle; B: With color Doppler. The deoxygenated blood from the vena cavae is directed to the mitral valve and thence into the left ventricle which is the pumping ventricle for the pulmonary artery and the pulmonary circulation. LV: Left ventricle; RV: Right ventricle.

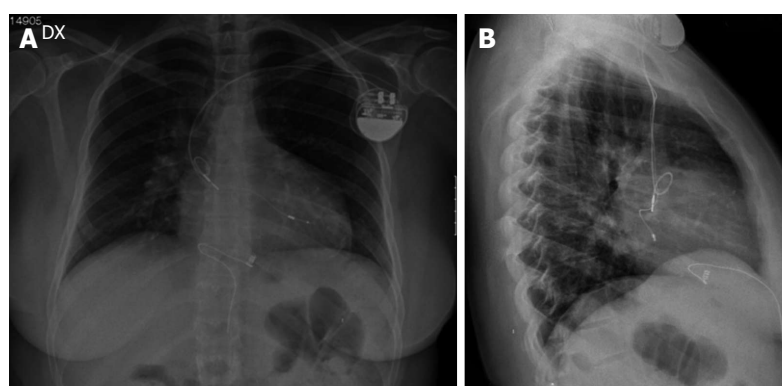


Figure 2 Chest X-ray. A: Antero-posterior chest X-ray after pacemaker implantation to confirm the position of the pacemaker leads. The ventricular lead is situated in the anatomic left ventricle, and the atrial lead in the systemic venous baffle; B: Lateral chest X-ray after pacemaker implantation to confirm the position of the pacemaker leads. In this patient the left atrial appendage was kept outside of venous tissue therefore the atrial lead was inserted and screwed into the systemic venous channel and a loop was created.

pericardial tissue and whether the electrode remains in the correct position.

We present the case of a young woman corrected with a Mustard procedure undergoing successful transvenous double chamber pacemaker implantation with the atrial lead placed in the systemic venous channel.

CASE REPORT

A 32-year-old female was born with a transposition of the great arteries (TGA), a large defect of the ventricular septum and a persistent ductus. At six months old she had a MO which involved closure of the defect of the ventricular septum and ductus arteriosus.

After the operation, she showed no symptoms at regular outpatient clinics. Nonetheless, 31 years after the MO she experienced dizziness, progressive tiredness, and shortness of breath. Echocardiography revealed a good left and right ventricular function (Figure 1).

With Holter monitoring we observed periods of atrioventricular junctional escape rhythm, high degree of atrioventricular block and pauses of up to 5.4 s. Indication was given for a pacemaker implantation. Due to the dizzy spells caused by sinus node dysfunction in addition

to atrioventricular conduction disturbances, the patient was subjected to a transvenous double chamber pacemaker implantation. Through the left cephalic vein an active-fixation electrode was introduced and placed in the apex of the anatomic left (subpulmonary) ventricle. Satisfactory values of sensing (8 mV) and pacing thresholds (0.5 mV) were gained without diaphragmatic stimulation.

In this patient the left atrial appendage was kept outside of venous tissue and therefore the passive-fixation atrial lead was inserted into the systemic venous channel and a loop was created.

Sensing and pacing thresholds were 1 mV and 2 V per 0.5 ms respectively. Post-procedural X-ray confirmed adequate positioning of the leads (Figure 2). The patient was discharged after an uneventful postoperative course.

At the 5-year follow up the leads were still in the correct position and sensing and pacing values were not subject to change. The woman was asymptomatic in sinus rhythm with a regular ventricular activation driven from the atrium.

DISCUSSION

TGA accounts for 5% to 7% of all congenital heart

Table 1 Potential technical and procedure-related complications of pacing following Mustard procedure

Complications
Lead dislodgement
Lead positioning
Abnormal anatomy post surgery
Spontaneous systemic venous baffle and venous obstruction
Systemic venous baffle and venous obstruction after lead insertion
Ventricular rhythm disturbances
Pacing thresholds, pacing impedance and sensing inadequate measurements or variations
System erosion/infection
Endocarditis risk
Paradoxical thromboembolic events

anomalies^[7]. The surgical repairs for TGA were first introduced by Senning in 1959; Mustard modified this technique in 1964^[1].

At the moment an anatomical correction is the most extensively used procedure; and the arterial switch has largely taken the place of the atrial switch procedure. Nonetheless late development of both atrial arrhythmias are well recognized late complications of atrial baffle surgery^[4-11] (Table 1).

Intra-atrial re-entrant tachycardia is the most common arrhythmia found among these patients, which has been associated with development of heart failure and death^[5,9].

In particular, causes of arrhythmias after the Mustard repair include^[4,12,13]: (1) damage during surgery to the sinus node or sinus node artery; (2) break of intra-atrial conduction by interruption of internodal pathways; and (3) intraoperative damage to atrioventricular (AV) node conduction tissue.

Pacemaker implantation is indicated for patients after MO who have a HR < 30 beats/min, Stokes-Adams episodes, patients requiring pharmacological therapy for tachyarrhythmias, or those with a poor systemic ventricular function and bradycardia^[14,15]. In addition, some MO patients require pacemaker implantation for sinus node dysfunction, AV block, in order to permit medical therapy of tachyarrhythmias or as an anti-tachycardia therapy^[16].

Pacemaker implantation in this setting can be technically challenging because of the complex anatomy^[17] and the possibility of complications such as systemic venous baffle obstruction or left innominate vein, right/left subclavian vein obstruction^[18]. Therefore, the determination of the exact vascular anatomy is mandatory to decide the most suitable position for placing the leads.

In this regard echocardiography, venography or intra-venous digital subtraction angiography before implantation may be of great help in studying the anatomy structural variations before pacemaker implantation.

However, usually one electrode is placed in the apex of the anatomic left (subpulmonary) ventricle and the atrial lead is fixed to the left atrial appendage^[7]. Berul *et al.*^[19] suggests, in the postoperative Mustard procedure,

that the superior aspect of the systemic venous-left atrium is the most optimal location.

Nonetheless, when left atrial appendage is not in place or it is not included into the systemic venous atrium, it is impossible to screw the atrial lead into the left atrial appendage. The electrode may be positioned in the systemic venous atrium but, as it consists partially of pericardial tissue, there are concerns associated with obtaining sub-optimal sensing and pacing thresholds and, despite this, there are no studies addressing the feasibility and efficacy of transvenous leads implanted into the pericardial baffle.

We present the case of a 32-year-old female undergoing a Mustard operation at six months of age who had transvenous double chamber pacemaker implantation because of high-degree atrioventricular block.

The ventricular electrode was placed in its usual position in the apex of the anatomic left (subpulmonary) ventricle avoiding creating a loop in this location which can be a substrate for ventricular ectopic beats^[4]. In contrast, since the left atrial appendage was outside the systemic venous atrium, it was impossible to place the lead into the left auricular appendix. Therefore, the atrial lead was positioned in the systemic venous channel and a passive fixation pacing was chosen to avoid pericardial baffle damage. Nonetheless, the use of passive-fixation pacing may lead to electrode dislodgement and this risk is raised by the absence of trabecular structures in the systemic venous channel differently from the left atrial appendage. Therefore, to prevent lead dislodgement, we created an electrode loop in the tube-like systemic venous channel.

At the end of the procedure sensing and pacing thresholds were adequate and, after 5 years, leads were still in the correct position with unchanged sensing and pacing thresholds.

In conclusion, the case of our patient demonstrates that in patients after Mustard repair, when the left atrial appendage is not reachable for surgical or anatomical reasons, the lead can be easily and safely placed in the systemic venous left atrium gaining satisfactory sensing and pacing thresholds and with no risk of lead dislodgement.

ACKNOWLEDGMENTS

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COMMENTS

Case characteristics

A 32-year-old female born with a transposition of the great arteries (TGA), a large ventricular septal defect and a patent ductus arteriosus who underwent a mustard operation (MO) at six months of age.

Clinical diagnosis

Progressive fatigue and dizziness and shortness of breath 31 years after her operation.

Differential diagnosis

Mobitz I second-degree atrioventricular (AV) block from Mobitz II second-degree AV block, as well as Mobitz II second-degree AV block from third-degree AV block.

Imaging diagnosis

Echocardiography, venography or intravenous digital subtraction angiography prior to implantation may be of great help in studying the anatomical structural variations before pacemaker implantation.

Pathological diagnosis

Holter monitoring showed episodes of atrioventricular junctional escape rhythm and high degree atrioventricular block.

Treatment

Indication was given for a pacemaker implantation.

Related reports

Some MO patients require pacemaker implantation for sinus node dysfunction, AV block, to permit medical therapy of tachyarrhythmias or as anti tachycardia therapy.

Term explanation

Mustard Operation is surgical treatment of TGA nowadays an anatomical correction is more preferred and this arterial switch procedure has largely replaced MO.

Experiences and lessons

The case presented demonstrates that, when the left atrial appendage is not included into the systemic venous atrium, the lead can be easily and safely placed in the systemic venous left atrium gaining satisfactory sensing and pacing thresholds despite it consists partially of pericardial tissue.

Peer review

A well-written case report merits consideration for publication as it describes a novel idea for permanent pacing in a patient with Mustard procedure.

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Acute myocarditis triggering coronary spasm and mimicking acute myocardial infarction

Andreas Kumar, Rodrigo Bagur, Patrick Béliveau, Jean-Michel Potvin, Pierre Levesque, Nancy Fillion, Benoit Tremblay, Éric Larose, Valérie Gaudreault

Andreas Kumar, Rodrigo Bagur, Patrick Béliveau, Jean-Michel Potvin, Pierre Levesque, Nancy Fillion, Benoit Tremblay, Valérie Gaudreault, Division of Cardiology, Department of Medicine, Quebec University Hospital Centre, Quebec, G1R 2J6, Canada

Andreas Kumar, Rodrigo Bagur, Patrick Béliveau, Jean-Michel Potvin, Pierre Levesque, Nancy Fillion, Benoit Tremblay, Valérie Gaudreault, Department of Medicine, Laval University, Quebec City, Quebec, G1R 2J6, Canada

Éric Larose, Quebec Heart and Lung Institute, Laval University, Quebec City, Quebec, G1V 4G5, Canada

Author contributions: Kumar A, Bagur R and Levesque P directly participated in the case, conception, design of study, acquisition of data and drafting the article; Kumar A, Bagur R, Béliveau P, Potvin JM, Levesque P, Fillion N, Tremblay B, Larose É and Gaudreault V contributed to manuscript writing, including revising it critically for important intellectual content; Kumar A and Bagur R contributed to supportive work, including technology and materials support; all authors approved the final version of the manuscript to be published and report no financial relationships or conflicts of interest regarding the content herein.

Correspondence to: Rodrigo Bagur, MD, PhD, FAHA, Attending Cardiologist, Interventional Cardiologist, Division of Cardiology, Department of Medicine, Quebec University Hospital Centre, 11 côte du Palais, L'Hotel-Dieu de Québec, Quebec City, Quebec, G1R 2J6, Canada. rodrigo.bagur@fmed.ulaval.ca
Telephone: +1-418-6915750 Fax: +1-418-6915415

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spasm is an uncommon association, it is important to recognize it, particularly for the management for those patients presenting with ST-segment elevation and suspect myocardial infarction and angiographically normal coronary arteries. The present report highlights the role of cardiovascular magnetic resonance imaging to identify acute myocarditis as the underlying cause.

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Key words: Myocarditis; Acute coronary syndrome; Coronary spasm; Myocardial infarction

Core tip: The present report highlights the role of cardiovascular magnetic resonance imaging to identify acute myocarditis as the underlying cause of coronary spasm presenting with ST-segment elevation myocardial infarction in a young healthy man.

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Abstract

A 24-year-old healthy man consulted to our center because of typical on-and-off chest-pain and an electrocardiogram showing ST-segment elevation in inferior leads. An urgent coronary angiography showed angiographically normal coronary arteries. Cardiovascular magnetic resonance imaging confirmed acute myocarditis. Although acute myocarditis triggering coronary

INTRODUCTION

Myocarditis has been frequently associated in patients with acute chest pain syndrome and angiographically normal coronary arteries^[1]. When the clinical presentation plus dynamic electrocardiographic (ECG) changes is quite suggestive of an acute coronary syndrome, coronary angiography is currently the first imaging diagnostic assessment in this setting. As a complementary imaging tool, cardiovascular magnetic resonance (CMR) imaging

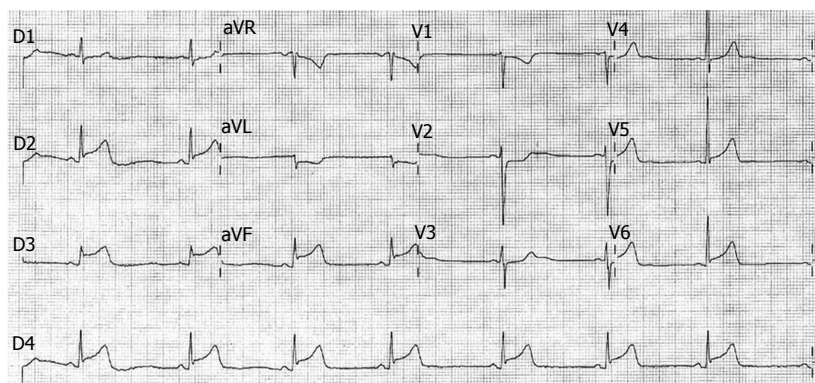


Figure 1 Twelve-lead electrocardiogram showing sinus rhythm with ST-segment elevation in the inferior leads and mirror image (mild ST-segment depression) in V1 to V3 and aVL.

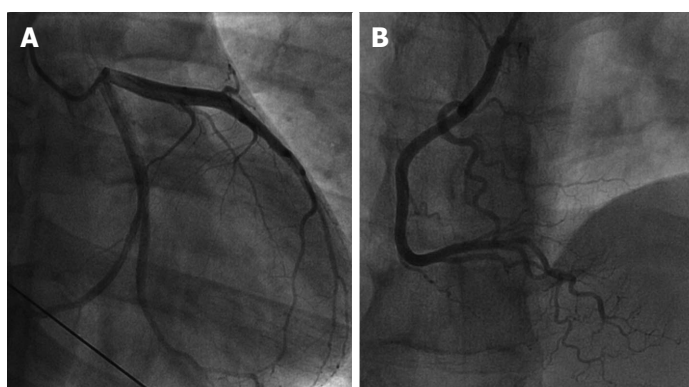


Figure 2 A and B coronary angiography showing angiographically normal coronary arteries.

provides a strong evidence for tissue characterization while completing the differential diagnosis.

CASE REPORT

A 24-year-old male consulted our emergency room complaining of 24 h of typical, intense on-and-off chest-pain. He had no previous medical history and no risk factors for coronary disease. There was no history suggesting a recent virus infection or drug use. During a chest pain episode in the emergency room, the ECG showed ST-segment elevation in the inferior leads (Figure 1). Troponin was positive on admission. An urgent coronary angiogram was performed showing angiographically normal coronary arteries (Figure 2), and the ECG normalized spontaneously. On the coronary care unit, 8-10 h after cardiac catheterization, the patient experienced a new episode of chest-pain with recurrence of inferior ST-segment elevation. A treatment with intravenous nitroglycerin was started which led to resolution of chest-pain and ST-segment normalization. Two-dimensional Doppler echocardiography showed a very mild infero-lateral hypokinesia with preserved left ventricular ejection fraction. Of note, the creatine kinase and the Troponin-I peaked at 1600 IU/L (normal value < 150 IU/L) and 51.8 micrograms/mL (normal value < 0.02 micrograms/mL) respectively, within 24 h. In order to characterize

the nature of this clinical scenario, the patient underwent CMR imaging confirming the mild infero-lateral hypokinesia (Figure 3A and B). In addition, tissue characterization showed myocardial edema localized in the epicardium of the lateral and infero-lateral walls (Figure 3C), the same area showed late gadolinium enhancement (Figure 3D). The subendocardial tissue appeared normal; therefore, highly compatible with acute myocarditis. The patient was discharged home seven days after admission on long acting Nifedipine and anti-inflammatory therapy.

DISCUSSION

Acute myocarditis triggering coronary vasospasm is a rare association. Especially myocarditis caused by Parvovirus B19, which affects endothelial cells, has been associated with coronary vasospasm^[2]. While coronary vascular smooth muscle cell dysfunction leading to Prinzmetal angina is an important differential diagnosis as well as coronary spasm on atherosclerotic coronary disease, myocarditis is an important but probably less frequent diagnosis to consider. The present report highlights the role of CMR imaging to identify acute myocarditis as the underlying cause. The epicardial distribution of edema and necrosis is a hallmark of myocarditis, as opposed to ischemic injury caused by epicardial coronary artery disease which necessarily leads to injury including the subendocardium^[3-6].

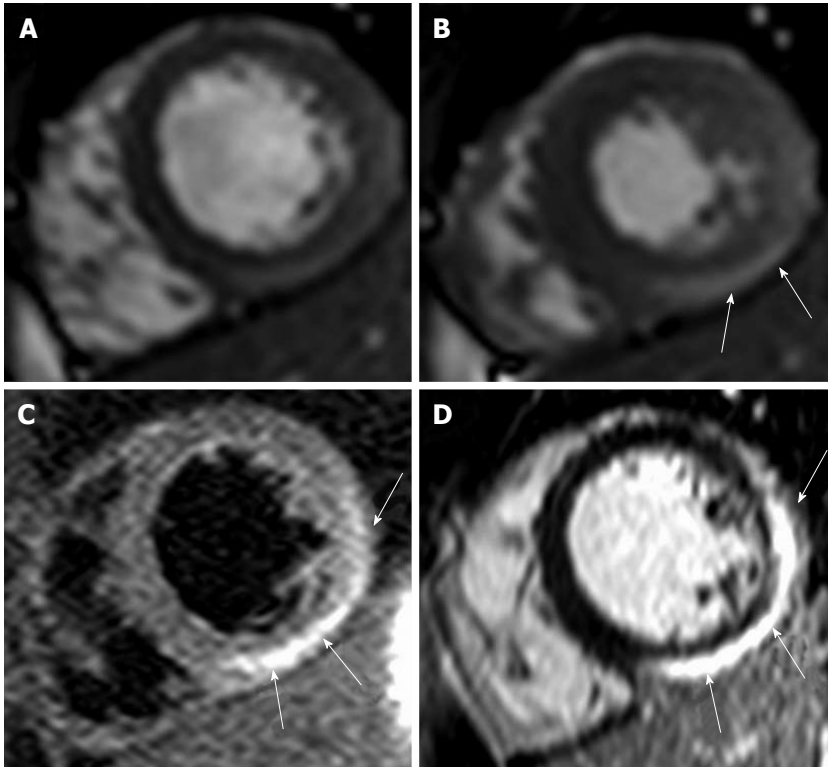


Figure 3 Cardiovascular magnetic resonance. Upper panel: Still frames of cine movies at end-diastole (A) and end-systole (B) showing mild infero-lateral hypokinesis (arrows); C: T2-STIR image showing myocardial edema in the lateral and inferior lateral epicardial wall (arrows); D: Gadolinium-enhanced image showing late enhancement predominately in the epicardial lateral and infero-lateral wall (arrows), highly compatible with acute myocarditis.

Myocarditis and epicardial coronary artery disease imply differences in medical treatment, therefore CMR enables the non-invasive assessment of changes in myocardial tissue composition (myocardial edema, hyperemia, and necrosis) and thus allowed for establishing the diagnosis of acute myocarditis^[3-6].

COMMENTS

Case characteristics

A healthy young man presenting with typical chest pain and an electrocardiogram showing inferior wall ST-elevation.

Clinical diagnosis

Acute myocardial infarction was the most likely clinical diagnosis given the description of chest pain and the electrocardiographic findings.

Differential diagnosis

Coronary vascular smooth muscle cell dysfunction leading to Prinzmetal angina as well as coronary spasm on atherosclerotic coronary disease are important differential diagnosis.

Laboratory diagnosis

Serial troponin levels progressively increased.

Imaging diagnosis

Coronary angiography demonstrated angiographically normal coronary arteries and cardiovascular magnetic resonance imaging showed myocardial edema localized in the epicardium of the lateral and infero-lateral walls, the same area showed late gadolinium enhancement.

Treatment

The patient was medically managed and discharged home seven days after admission on long acting Nifedipine and anti-inflammatory therapy.

Related reports

Myocarditis caused by Parvovirus B19, which affects endothelial cells, has been associated with coronary vasospasm.

Experiences and lessons

Although an uncommon association, it is important to recognize it, particularly for the management of those patients presenting with typical chest pain and electrocardiographic ST-segment elevation and therefore mimicking myocardial infarction.

Peer review

The authors present a case that reports an uncommon association of an acute myocarditis triggering coronary spasm and presenting as ST-elevation myocardial infarction. The manuscript is clearly written, well organized, comprehensive, appropriate referenced and concise in its content.

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Contents

Monthly Volume 6 Number 10 October 26, 2014

TOPIC HIGHLIGHT

- 1049 High-density lipoprotein and atherosclerosis: Roles of lipid transporters
Uehara Y, Saku K
- 1060 Contribution of cardiovascular magnetic resonance in the evaluation of coronary arteries
Mavrogeni S, Markousis-Mavrogenis G, Kolovou G
- 1067 ST-segment elevation: Distinguishing ST elevation myocardial infarction from ST elevation secondary to nonischemic etiologies
Deshpande A, Birnbaum Y

REVIEW

- 1080 Non-interventional management of resistant hypertension
Doumas M, Tsioufis C, Faselis C, Lazaridis A, Grassos H, Papademetriou V
- 1091 Mitochondria-targeted agents: Future perspectives of mitochondrial pharmaceuticals in cardiovascular diseases
Ajith TA, Jayakumar TG

MINIREVIEWS

- 1100 Perioperative clinical variables and long-term survival following vascular surgery
Garcia S, McFalls EO
- 1108 Quantitative assessment of myocardial blush grade in patients with coronary artery disease and in cardiac transplant recipients
Hofmann NP, Dickhaus H, Katus HA, Korosoglou G

OBSERVATIONAL STUDY

- 1113 Neuroticism personality trait is associated with Quality of Life in patients with Chronic Heart Failure
Samartzis L, Dimopoulos S, Manetos C, Agapitou V, Tasoulis A, Tseliou E, Pozios I, Kaldara E, Terrovitis J, Nanas S

CASE REPORT

- 1122 Aorto-right atrial fistula: Late complication of tricuspid valve infective endocarditis
Villablanca PA, Sukhal S, Maitas O, Onuegbu A, Muñoz-Peña JM, Joseph A, Requena C, Mohananey D

- 1127** Electrical storm in systemic sclerosis: Inside the electroanatomic substrate
Casella M, Carbucicchio C, Russo E, Pizzamiglio F, Golia P, Conti S, Costa F, Dello Russo A, Tondo C
- 1131** Anaphylactic cardiovascular collapse during hemodialysis: Kounis syndrome in the dialysis room
Mazarakis A, Bardousis K, Almpanis G, Mazaraki I, Ouzounis A, Kounis NG

Contents

World Journal of Cardiology
Volume 6 Number 10 October 26, 2014

APPENDIX I-V Instructions to authors

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WJC 6th Anniversary Special Issues (1): Hypertension

High-density lipoprotein and atherosclerosis: Roles of lipid transporters

Yoshinari Uehara, Keihiro Saku

Yoshinari Uehara, Keihiro Saku, Department of Cardiology, Fukuoka University School of Medicine, Fukuoka 814-0180, Japan
 Author contributions: Uehara Y designed and wrote the manuscript; Saku K was involved in editing the manuscript.

Correspondence to: Yoshinari Uehara, MD, PhD, Department of Cardiology, Fukuoka University School of Medicine, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan. ueharay@fukuoka-u.ac.jp

Telephone: +81-92-8011011 Fax: +81-92-8652692

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Abstract

Various previous studies have found a negative correlation between the risk of cardiovascular events and serum high-density lipoprotein (HDL) cholesterol levels. The reverse cholesterol transport, a pathway of cholesterol from peripheral tissue to liver which has several potent antiatherogenic properties. For instance, the particles of HDL mediate to transport cholesterol from cells in arterial tissues, particularly from atherosclerotic plaques, to the liver. Both ATP-binding cassette transporters (ABC) A1 and ABCG1 are membrane cholesterol transporters and have been implicated in mediating cholesterol effluxes from cells in the presence of HDL and apolipoprotein A-I, a major protein constituent of HDL. Previous studies demonstrated that ABCA1 and ABCG1 or the interaction between ABCA1 and ABCG1 exerted antiatherosclerotic effects. As a therapeutic approach for increasing HDL cholesterol levels, much focus has been placed on increasing HDL cholesterol levels as well as enhancing HDL biochemical functions. HDL therapies that use injections of reconstituted HDL, apoA-I mimetics, or full-length apoA-I have shown dramatic effectiveness. In particular, a novel apoA-I mimetic peptide, Fukuoka University ApoA-I Mimetic Peptide, effectively removes cholesterol *via* specific ABCA1 and other transporters, such as ABCG1, and has an an-

tiatherosclerotic effect by enhancing the biological functions of HDL without changing circulating HDL cholesterol levels. Thus, HDL-targeting therapy has significant atheroprotective potential, as it uses lipid transporter-targeting agents, and may prove to be a therapeutic tool for atherosclerotic cardiovascular diseases.

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Key words: ATP-binding cassette transporter; ATP-binding cassette A1; ATP-binding cassette G1; Apolipoprotein A-I; High-density lipoprotein; High-density lipoprotein therapy; apoA-I mimetic peptide; Reconstituted high-density lipoprotein

Core tip: The reverse cholesterol transport pathway played with high-density lipoprotein (HDL) has several potential antiatherogenic properties. Both ATP-binding cassette (ABC) A1 and ABCG1 are lipid transporters and have been involved in mediating cholesterol effluxes from cells in the presence of HDL or apoA-I, and they exerted antiatherosclerotic effects. As a therapeutic approach for increasing HDL cholesterol levels, much focus has been placed on increasing not only HDL cholesterol levels, but also HDL-biological functions. Reconstituted HDL and apoA-I mimetics have significant atheroprotective potential, as it uses lipid transporter-targeting agents, and may prove to be a novel therapeutic tool for atherosclerotic cardiovascular diseases.

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INTRODUCTION

High-density lipoprotein (HDL) cholesterol is widely

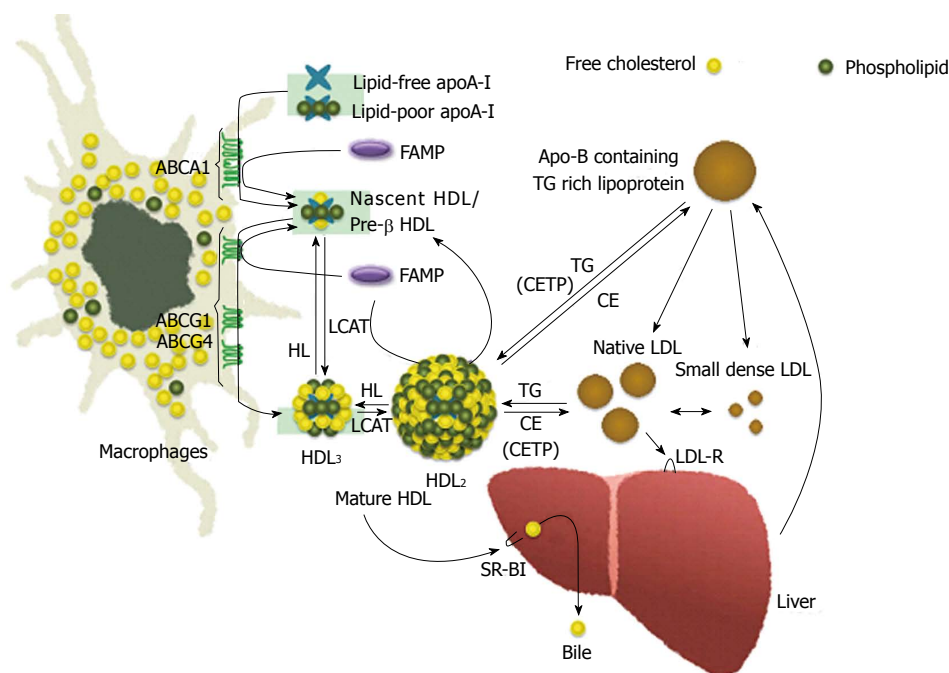


Figure 1 Illustration of high-density lipoprotein metabolism and suggested function of fukuoka university ApoA-I mimetic peptide in high-density lipoprotein metabolism. ABC: ATP-binding cassette transporter; TG: Triglyceride; CE: Cholesteryl ester; CETP: Cholesteryl ester transfer protein; HL: Hepatic lipase; apo: Apolipoprotein; HDL: High-density lipoprotein; FAMP: Fukuoka university ApoA-I mimetic peptide; CETP: CE transfer protein; SR-BI: Scavenger receptor BI; LDL: Low-density lipoprotein; LCAT: Lecithin cholesterol acyltransferase; LDL-R: Low-density lipoprotein receptor; SR-BI: Scavenger receptor class B, type I; FAMP: Fukuoka University ApoA-I mimetic peptide.

known as “good cholesterol”, because various previous studies have found a negative correlation between the risk of cardiovascular events and serum HDL cholesterol levels^[1]. However, this is still controversial whether the association is the cause or just only an ensuing symptom of a general atherosclerotic damage. HDL has several potential for antiatherogenic properties, for instance, cholesterol is transported from peripheral tissues such as the cells in the arterial walls to the liver by HDL particles, where it is used for a composition of lipoproteins and a synthesis of bile acids, steroid hormones, or fat-soluble vitamins^[1]. Whereas, low-HDL cholesterolemia is often observed as a characterized component of metabolic syndrome, such as in people who are overweight or obese, those with glucose intolerance or have obvious diabetes, those with hypertriglyceridemia, and those with high blood pressure, each of which conditions contribute to the cause of atherosclerosis^[2].

METABOLISM AND THE FUNCTIONS OF HDL

Although HDL is a lipoprotein when isolated by ultracentrifugation has a density in the range of 1.063-1.21 g/mL (HDL₂, 1.063 < d < 1.125 g/mL; HDL₃, 1.125 < d < 1.21 g/mL), HDL composes a heterogeneous group of particles that differ in density, size, composition of apolipoprotein (apo) or lipid, and electrophoretic mobility^[3]. It is possible to separate HDL into two major subfractions on the basis of electro-mobility by electrophoresis; the major subfraction has the same mobility as alpha HDL,

whereas the other subfractions migrate similar to pre-beta HDL, in addition the majority of HDL particles in human plasma are alpha HDL, and pre-beta HDL represents only 2%-14% of all apoA-I^[4,5] (Figure 1).

HDL metabolism has the complicated mechanisms in association with several HDL-related genes such as various enzymes and protein, lipids, receptors, or transporters and its synthesis involves a complex pathway. The underlying genetic deficiency in many cases of primary low-HDL cholesterolemia are not clearly understood, however mutations in three pivotal genes as apoA-I, lecithin: cholesterol acyltransferase, and ATP-binding cassette transporter (ABC) A1, are associated with reducing serum HDL cholesterol levels, furthermore some of these genes' mutations are also closely correlated with an increased risk of premature atherosclerosis and coronary artery disease (CAD)^[6].

TANGIER DISEASE, A FAMILIAL HDL DEFICIENCY

Tangier disease (TD) is the most severe form of HDL deficiency, which was first described by Fredrickson *et al*^[7]. The biological hallmarks of TD patients' plasma are a defect of HDL cholesterol, reduced low-density lipoprotein (LDL) cholesterol levels, and moderate increased triglyceride. The plasma apoA-I concentration in TD is markedly decreased to approximately 1%-3% of normal. TD is a very rare autosomal recessive disorder which is characterized by the almost absence serum apoA-I and HDL cholesterol levels. Furthermore, cholesteryl ester (CE) accumulates in many macrophage enriched tissues, such

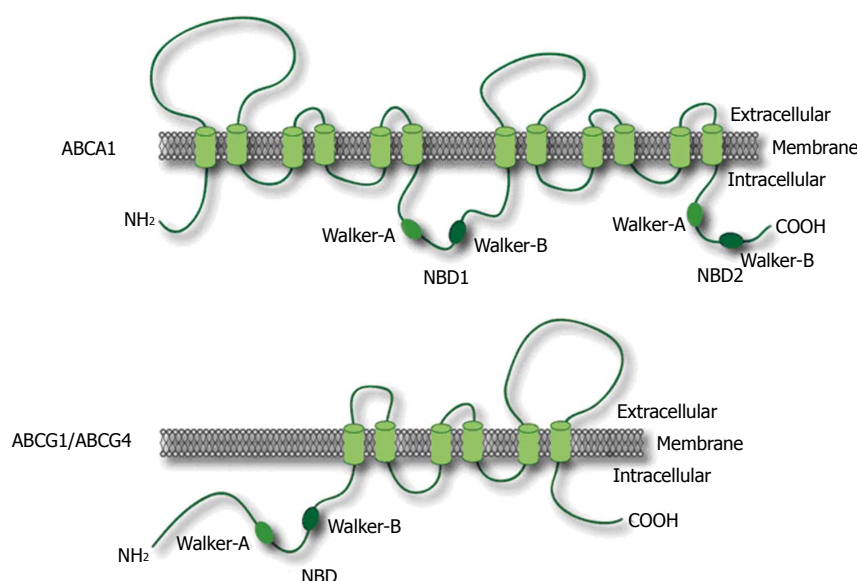


Figure 2 Secondary structures of the ATP-binding cassette transporter A1, ATP-binding cassette G1 and ATP-binding cassette G4 transporters. The ATP-binding cassette transporters (ABC)A1 transporter comprises 2201 amino acids with two transmembrane domains comprising two nucleotide binding domains (NBD-1 and -2) and six transmembrane helices, which contain two conserved peptide motifs, Walker-A and -B. ABCA1 is characterized as two large extracellular loops and N-terminus oriented towards the cytosol. Both ABCG1 and ABCG4 proteins have one transmembrane domain comprising six transmembrane helices and one NBD that contains two conserved peptide motifs, Walker-A and Walker-B.

as tonsils, spleen, liver, lymph nodes, peripheral nerves, thymus, and also arterial walls. Clinical symptoms among homozygotes patients include hepatosplenomegaly, hyperplastic orange-yellow tonsils, corneal opacification, and premature CAD and atherosclerosis in a half of cases as well as relapsing peripheral neuropathy due to CE deposition in macrophages and Schwann cells^[7-9].

In 1999, a cause of TD was found in a defect of the ABCA1 (formerly *ABCI*) gene^[1,10,11] that is located on chromosome 9q31. This gene comprises 50 exons that span a region of approximately 149 kb^[12,13]. ABCA1 has been identified as an important gene for regulating cellular cholesterol homeostasis and serum HDL cholesterol levels, which is defect in patients with TD. *ABCA1* gene mutations cause gene dose-dependent decreases in serum HDL cholesterol levels and a decreased capacity of skin fibroblasts and monocyte-derived macrophages releasing cholesterol in the presence of extracellular apolipoproteins in TD patients and their heterozygous relatives^[1,10,11,14,15].

A transmembrane protein, ABC transporter facilitates to carry out the specific substrates across cell membranes in an ATP-dependent manner. ABCA1 is a member of the ABC transporter superfamily comprised 48 human transporters, and the superfamily is divided into seven subfamilies, including from half- to full-transporters, designated ABCA-ABCG. These transporters are integral membrane proteins carrying out various substrates, including lipids, ions, peptides, amino acids, carbohydrates, vitamins, glucuronides, glutathione conjugates, and xenobiotics^[16,17]. ABCA1 is expressed in various organs in human, particularly the highest expression levels are existed in the placenta, liver, lung, adrenal glands, and fetal tissues^[18].

ABC transporter superfamilies are defined by the presence of similar nucleotide binding domains (NBD) to interact with ATP. These domains have two conserved peptide motifs, Walker-A and Walker-B, which are found in many proteins that utilize ATP^[16,19] (Figure 2).

ABC TRANSPORTER ROLES IN HDL METABOLISM

ABCA1 transporter functions and their relationships with HDL metabolism

ABCA1 proteins transport phospholipids (PLs) and cholesterol from the membranous inner leaflet to the outer leaflet, subsequently lipid-poor or lipid-free apoA-I takes up this transported cholesterol and PLs by ABCA1 to form nascent HDL^[20]. ABCA1 is localized at the plasma membrane and intracellular compartments, where it can potentially facilitate lipid transport to either cell surface-bound^[21] or internalized apolipoproteins^[22].

HDL metabolism is composed of at least three different steps. As the first step, lipid-free or lipid-poor apoA-I removes free cholesterol from peripheral cells through ABCA1 transporter to form nascent-HDL. Second, nascent-HDL has a further lipidation, thereafter it grows to mature-HDL. Third, mature-HDL interacts with other apoB containing lipoproteins, such as intermediate density lipoprotein (IDL) and very-low-density lipoprotein (VLDL). Thus, ABCA1 is indispensable for the nascent-HDL formation, in addition it is also an important and essential molecule for the initial step of the reverse cholesterol transport (RCT).

Cultured blood monocyte-derived (mod)-macrophages from a healthy subject showed an approximately 125% increase in cholesterol efflux mediated lipid-free apoA-I, whereas it did not respond to apoA-I mediated efflux in macrophages from TD patients^[23]. Although a lipid-free apoA-I showed an increase the cholesterol efflux mediated by in cultured mod-macrophages from healthy persons, the apoA-I did not elevate cholesterol efflux in mod-macrophages from TD patients. These results indicated that ABCA1 is a key molecule for apoA-I-specific cholesterol efflux pathway, but not basal efflux in macrophages.

Since ABCA1 plays an important role in mediat-

ing cholesterol and PL effluxes by lipid-free apoA-I, it is involved in a formation of discoidal HDL precursor, furthermore ABCA1 poorly interacts with HDL2 and HDL3. Patients with TD have extremely low levels of HDL cholesterol and they cannot compose nascent HDL particles due to a genetic defect in *ABCA1* gene.

Disrupting the ABCA1 in mice resulted in HDL deficiency and impaired cholesterol transport similar to TD^[24,25]. ABCA1 overexpression resulted in increased apoA-I-mediated cholesterol efflux in transgenic mice^[26,27]. These results indicate that ABCA1 is an important gene in regulating circulating HDL cholesterol levels and cellular cholesterol homeostasis.

ABCG1 transporter functions and their relationships to HDL metabolism

ABCG1, formerly ABC8 is also a member of the ABC transporter family which has been mapped on chromosome 21q22.3^[19,28-32]. ABCG1 is one of half-transporter that contains only one NBD and a transmembrane domain, in contrast to ABCA1^[19,31] (Figure 2). Thus, ABCG1 may require a dimeric partner to become active with ABCG1 or ABCG4.

Although ABCA1 promotes cholesterol efflux to lipid-poor or lipid-free apoA-I, it only modestly induces lipid efflux of smaller particles, such as HDL₃, and does not promote a cholesterol efflux of the larger HDL₂ fraction^[33,34]. It has been also shown by Wang *et al.*^[35] that ABCG1 and ABCG4 contributed to HDL₂- and HDL₃-mediated cholesterol effluxes and had an important function related to HDL lipidation^[35-37].

Administering a high-cholesterol, high-fat diet to ABCG1 knock-out mice resulted in a large amount of lipid accumulation in macrophages, whereas overexpression of human *ABCG1* gene was able to protect a dietary fat-induced lipid accumulation in murine model^[38]. Moreover, It was shown by Mauldin *et al.*^[39] that reduced function of ABCG1 facilitated foam cell formation in diabetes mice^[39]. Transplanting bone marrow from ABCG1-deficient (*ABCG1*^{-/-}) mice into LDL receptor-deficient mice, a model of familial hypercholesterolemia, produced contrasting effects on the formation of atherosclerotic lesion^[40-42]. In contrast to these report, decreased lesion size and formation were observed in the absence of macrophages from ABCG1-deficient mice^[41,42], and whole body ABCG1 expression protected against the development of early atherosclerotic plaque^[43]. However, it remains unclear that the physiological roles of ABCG1 and its contribution to atherosclerotic progression in humans. In addition, ABC transporters such as ABCG1 and ABCG4, but not ABCA1, are not only responsible for passive and nonspecific efflux pathway but also mature HDL-mediated cholesterol efflux, which are spherical and transport almost all HDL cholesterol^[35,37].

ROLES OF ABCG5 AND ABCG8 TRANSPORTER

ABCG5 and ABCG8 are half-transporters as well as

ABCG1 that function together as a heterodimer, and mutations in either of these genes can cause sitosterolemia which is a rare autosomal, recessively inherited disorder, characterized by premature atherosclerosis and xanthomas^[44-47]. These transporters mediate the sterols efflux including cholesterol and plant sterols from enterocytes return into the intestinal lumen and their excretion into the bile^[44,48]. Accordingly, they protect the lipid accumulation in the body and augment RCT system. In animal model, *ABCG5* and *ABCG8* deficient mice have been shown to reduce a secretion of cholesterol in the bile and elevate sterol absorption^[49], on the other hand *ABCG5* and *ABCG8* genes-overexpressed mice promotes cholesterol secretion in the bile, decreases cholesterol absorption from diet, and increases neutral sterol excretion in the feces^[50]. Liver X receptor (LXR) agonists promote the cholesterol efflux by the upregulation of ABCA1 and ABCG1, and also stimulate ABCG5 and ABCG8 which accelerate direct HDL transport of intestine into the lumen, thus these genes also play an important role in the RCT system and their enhancement by LXR agonists prevent an atherosclerotic development^[51].

MECHANISMS OF ABCA1 AND ABCG1 GENE REGULATION

ABCA1 gene expression and cellular efflux of cholesterol are enhanced by cholesterol^[15,18], oxysterols^[52], retinoids^[53], and cAMP analogs^[15,54]. The *ABCA1* gene promoter has been analyzed^[13,52]. Both oxysterols and retinoids are ligands for the nuclear transcription factor, LXR α/β and retinoid X receptor-alpha (RXR α), respectively, which have been identified as an enhancer of *ABCA1* gene expression^[52,53,55,56]. It is present in dimeric form of LXR and RXR as active transcriptional heterodimers that preferentially bind to responsive elements in the ABCA1 gene promoter^[13,57]. LXR α/β and RXR α bind to the specific responsive element, called direct repeat 4 (DR4) element within the ABCA1 promoter, which is characterized by two direct hexameric repeats separated by four nucleotides, thereafter they are activated by oxysterols and retinoids^[58,59]. ABCA1 transcription are activated to bind either one or both ligands. Treatment with either a ligand of LXR α/β or RXR α enhances cellular ABCA1 expression, furthermore their combination treatment has a marked synergistic effect^[60].

Since peroxisome proliferator activating receptor (PPAR)- α and - γ agonists such as fibrates and thiazolidine derivative (TZD) upregulate LXR mRNA expression, the activation of PPARs indirectly enhances a transcription activity of ABCA1 *via* LXR in cultured cells. In contrast, it is already known the zinc finger protein ZNF202 transcription factor as a major transcriptional repressor for ABCA1. In addition to the factor ZNF202, unsaturated fatty acids, but not saturated one, drastically suppress ABCA1-mediated cholesterol effluxes from macrophages by which they antagonize the binding of specific agonist, oxysterol to LXR^[61,62]. Moreover, various transcription factors, such as upstream stimulatory factor (USF)1, USF2, Fra2, and Sp3, also have

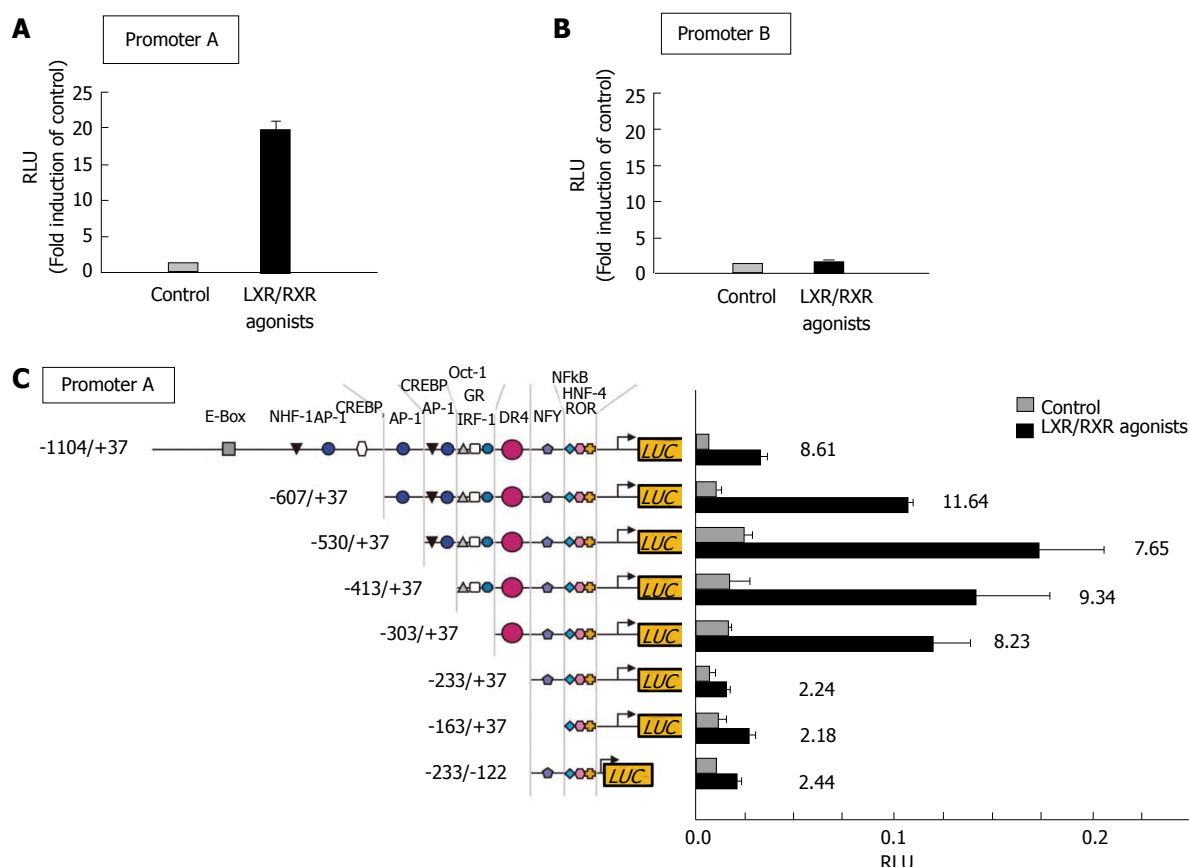


Figure 3 Response of liver X receptor and retinoid X receptor agonists to human ATP-binding G1 promoter activities in RAW264 cells. A: Human wild-type ATP-binding cassette transporter G1 (ABCG1) promoter-A located upstream of exon 1; B: Human wild-type ABCG1 promoter-B located upstream of exon 5; C: ABCG1 promoter (promoter-A; upstream of exon 1) vectors that contain a truncated 5'-region of the *ABCG1* gene. After transfection, cells were incubated with or without agonists of LXR [22(R)-hydroxycholesterol, 10 μ mol/L] and RXR (9-cis-retinoic acid, 10 μ mol/L). Results are expressed as mean \pm SD. Graphs modified from the paper by Uehara *et al.*^[62]. LXR: Liver X receptor; RXR: Retinoid X receptor; RLU: Relative luciferase units.

the potential to repress the *ABCA1* transcription^[63].

The *ABCG1* gene has a promoter upstream of exon 1 and another intron promoter, which encodes several transcripts^[64-66]. Our previous study demonstrated that LXR activation drastically increased the *ABCG1* promoter activity (Promoter-A) located upstream of exon 1 as well as the *ABCA1* gene (Figure 3A). On the other hand, the activity of *ABCG1* promoter-B located within intron 4 was not changed by an activation of LXR (Figure 3B)^[62]. These results indicate that the gene transcription of exon 5 and subsequent exons might be also regulated, at least in part, by the *ABCG1* promoter-A.

Electrophoretic mobility shift assay was done to confirm these findings, and it showed the existence of DNA-binding nuclear receptors on extracted *ABCG1* promoter-A having DR4 element. As would be expected from these finding, only the *ABCG1* promoter-A contained a DR4 element, but not promoter-B, which is required for binding to LXR α /RXR. In fact, a promoter response to ligands of LXR/RXR was totally abolished in the mutated *ABCG1* promoter lacked an active DR4 element^[62] (Figure 3C).

ABCG1 SINGLE NUCLEOTIDE POLYMORPHISMS

It remains unclear whether *ABCG1* itself contributes

to circulating lipid levels, such as HDL cholesterol and arterial plaque regression in humans. There have been only five reports on *ABCG1* polymorphisms. Our previous study was the first regarding an *ABCG1* polymorphism, which appeared to be a potent functional *ABCG1* polymorphism located in the promoter region^[67-71]. The *ABCG1* promoter -257T>G polymorphism, rs1378577, -394 T/G from the transcription start site (NM_207627.1: c. -394T>G), -134 T/G from exon 1 (NM_207627.1) is a single nucleotide mutation (SNP) on the *ABCG1* promoter region upstream of exon 1, which was reported to be a functional promoter with an LXR-responsive element^[62,67].

To investigate whether this promoter polymorphism influenced gene transcriptional activity, *in vitro* luciferase reporter gene assays were performed after transient transfection in cultured cells. In these experiments, the amount of luciferase activity was 25.7% higher in T allelic sequence containing construct than that in G allelic one on *ABCG1* promoter-A; these responses were significantly different (Figure 4A). *ABCG1* promoter activity induced by LXR and RXR agonists increased by 4.6-fold, and the amount of luciferase produced by the construct containing the T allelic sequence was 30.9% higher than that produced by the construct containing the G allele, which was also significantly different as well as in the absence of LXR/RXR agonists (Figure 4B). The transcription activity

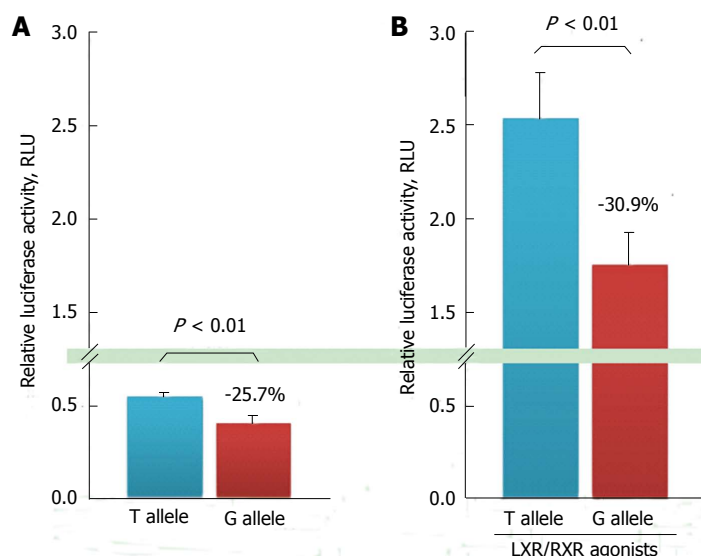


Figure 4 *In vitro* promoter activity assay for ATP-binding G1 promoter-A. ATP-binding cassette transporter G1 (ABCG1) promoter construct with a -257T/G mutation, -394 T/G from the transcription start site (NM_207627.1: c.-394T > G) on ABCG1 promoter-A, which is reported to be a functional promoter with an LXR-responsive element. A: ABCG1 transcription activity on a construct that contains the T or G allelic sequence; B: ABCG1 transcription activity induced by 5 μ mol/L of T0901317 (T0) and 9-cis-retinoic acid (9cisRA) on constructs that contain the T or G allelic sequence. Results are expressed as mean \pm SD. Graphs are modified from the paper by Furuyama *et al.*^[67]. ABC: ATP-binding cassette; RXR: Retinoid X receptor; LXR: Liver X receptor.

in the T allelic sequence was significantly higher than that in the G allelic sequence on ABCG1 promoter-A.

Furthermore, the ABCG1 promoter showed increased activity *via* stimulation by LXR and RXR, and a similar genotype-dependent effect on ABCG1 gene transcription under these conditions was identified. These results suggest that the ABCG1 promoter polymorphism might be an isolated regulating factor for *ABCG1* gene transcription activity, independent of LXR and RXR.

We genotyped 109 Japanese male CAD patients for the ABCG1 promoter SNP. This polymorphism was associated with CAD severity in Japanese men, but not with changes in lipid levels under fasting conditions in a case control study. Logistic regression analysis showed that there was an interaction between the ABCG1 promoter genotype and CAD severity.

Genotype frequencies were grouped on the basis of whether patients had multi- or single-vessel CAD. The adjusted relative risk associated with the G allele (assuming an additive effect) in a matched-pair analysis was 2.1 for multi-vessel CAD compared with single-vessel CAD and 3.5 for the G/G and T/G genotypes compared with T/T (assuming a dominant effect of the G allele)^[67]. These results were consistent with the proposition that the variations for *ABCG1* gene might make a contribution to interindividual variability in susceptibility or severity of atherosclerotic changes.

ABCG1 expression levels in atherosclerotic tissues might be lower among those with the G allele and may be associated with a mechanism for an increased incidence of atherosclerosis in these individuals. These results were similar to a previous study by Baldán *et al.*^[72] of transgenic mice in whom the *ABCG1* gene was deleted^[73]. Furthermore, a recent study regarding ABCG1 as a candidate gene with possible important antiatherogenic properties also illustrates the current interest in this transporter.

HDL-TARGETING THERAPY FOR ATHEROSCLEROSIS

Inhibiting scavenger receptor BI (SR-BI), CE transfer

protein (CETP) or PL transfer protein, and an activating ABCA1 or apoA-I elevate HDL cholesterol levels. However, it is uncertain whether the effects of these interventions on atherosclerosis are consistent with the results of studies with animal models and inborn human HDL metabolism errors. Although it has not found a such small molecule which strongly promotes apoA-I production, one possible candidate molecule is LXR agonist which increase HDL cholesterol levels *via* upregulation of ABCA1 and ABCG1 expressions. Unfortunately, previous study has shown that concurrent with an activation of RCT, the agonist induces hypertriglyceridemia consequent on increasing hepatic VLDL production.

As a therapeutic approach for increasing HDL levels, much research has focused both increasing HDL cholesterol levels and on enhancing HDL biochemical functions. HDL therapies that used injections of reconstituted HDL, apoA-I mimetics, or full-length apoA-I are remarkably effective^[74,75]. Nissen *et al.*^[75] showed that in humans, intravenous administration of ETC-216, an apoA-I-Milano complexed with phospholipids, produced a significant regression of coronary atherosclerotic plaques as determined by intravascular ultrasound (IVUS). After infusing ETC-216, regression of coronary atherosclerosis was accompanied by reverse remodeling of the external elastic membrane and with no changes in luminal dimensions as assessed by IVUS analyses^[76].

Reconstituted HDL (rHDL), a complex of apoA-I or apoA-I mimetics with PL, must be shaped as disc, and it may be a suitable administration in patients with atherosclerotic plaque and TD. ABCA1 plays an important role for apoA-I-mediated cholesterol efflux in macrophages, and thereby is involved in discoidal HDL precursor formation. Mature HDL particles shaped spherical induce cholesterol effluxes by other transporters such as ABCG1 and ABCG4, rather than ABCA1^[35]. We previously established a discoidal rHDL, which was a complex of human serum-derived full length of apoA-I with PL, 1-palmitoyl-2-oleoylphosphatidylcholine (POPC)^[77]. Interestingly, the apoA-I complex with a PL, a POPC/apoA-I disc, could take up cholesterol from macrophages in both nor-

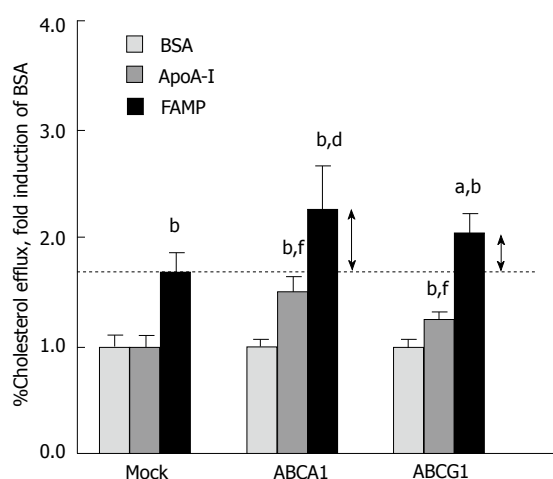


Figure 5 Fukuoka University apoA-I mimetic peptide effects on cellular cholesterol effluxes in cells that express ATP-binding A1 and ATP-binding G1. COS-7 cells were transiently transfected with an empty vector (mock) or with human ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1) cDNAs. Cholesterol efflux was determined after incubation with apoA-I or FAMP. Results are expressed as mean \pm SD. ^a $P < 0.05$ vs FAMP in mock; ^b $P < 0.01$ vs BSA; ^c $P < 0.01$ vs FAMP in mock; ^d $P < 0.01$ vs apoA-I in mock. Graph modified from the paper by Uehara *et al.*^[82]. FAMP: Fukuoka University ApoA-I mimetic peptide; BSA: bovine serum albumin; apoA-I: apolipoprotein A-I; FAMP: Fukuoka University ApoA-I Mimetic Peptide.

mal subjects and TD patients.

Although studies on the use of apoA-I mimetic peptides (e.g., 4F and L37pA) are underway^[78-80], none of these agents are currently available for clinical use. To develop a physiological HDL-generating apoA-I mimetic peptide that functions with ABCA1 transporter, different candidate peptides were synthesized by focusing on the amino acid sequence alignments of human apoA-I interactions with ABCA1. We recently established a novel short apoA-I mimetic peptide that comprised 24 amino acids and without phospholipids Fukuoka University ApoA-I Mimetic Peptide (FAMP), which retained the amphipathic helical structure of the 243-amino acid apoA-I and the ability to associate with lipids^[81]. This was shown to enhance HDL function and suppress aortic plaque formation in apoE-knockout mice that were fed a high-fat diet. FAMP markedly increased pre-beta HDL formation as well as increased the overall cholesterol effluxes from peripheral tissues^[82].

In contrast to apoA-I, FAMP-mediated cholesterol effluxes were not completely abolished under ABCA1-inactivated conditions, such as in cells treated with probucol, an ABCA1 antagonist, and Tangier macrophages. These results suggested that FAMP functioned in removing cholesterol through both the ABCA1 pathway and another specific pathway that must be dependent on ABCG1 transporters (Figure 1). In support of this, COS-7 cells that were transiently transfected with the *ABCA1* and *ABCG1* genes had significantly increased FAMP-mediated effluxes compared with mock transfection (Figure 5).

Injections of HDL apoA-I mimetics, apoA-I-Milano, and full-length apoA-I are effective both *in vitro* and *in*

vivo. However, it remains unclear whether apoA-I or its mimetics actually enter atherosclerotic plaque lesions and remove cholesterol. ApoA-I may generate nascent, new HDL and reverse the macrophage foam cell phenotype.

We developed a novel PET tracer that was functionalized with DOTA and labeled with ⁶⁸Ga to specifically image the status of atherosclerotic plaques. Atherosclerotic plaques and aortic atherosclerotic plaques show high uptake of this tracer, and this novel tracer provides for impressive *in vivo* imaging of an aortic plaque using PET/CT^[83]. HDL-targeting therapy, including FAMP, may have tremendous atheroprotective potential and prove to be a new therapeutic tool for atherosclerotic cardiovascular disease. While most research has focused on the therapeutic use of HDL, an apoA-I mimetic peptide may also contribute to the development of a tool for plaque diagnosis.

CONCLUSION

The RCT pathway has several potential antiatherogenic properties. Both ABCA1 and ABCG1 are lipid transporters on plasma membrane that have been contributed in mediating effluxes of cholesterol and PLs from cells in the presence of lipid-poor or lipid-free apoA-I and HDL. As a therapeutic approach for increasing HDL levels, much research has focused both on increasing HDL cholesterol levels and on enhancing HDL biochemical functions. HDL therapies with reconstituted HDL, apoA-I mimetics, or full-length apoA-I are dramatically effective. In particular, a novel apoA-I mimetic peptide, FAMP, effectively removes cholesterol *via* specific ABCA1 and other transporters, such as ABCG1. FAMP has an antiatherosclerotic effect by enhancing biological HDL functions without changing circulating HDL cholesterol levels. These HDL-targeting therapies have significant atheroprotective potential, as they are lipid transporter-targeting agents. Thus, HDL-targeting therapy may prove to be a therapeutic tool for atherosclerotic cardiovascular diseases.

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WJC 6th Anniversary Special Issues (2): Coronary artery disease

Contribution of cardiovascular magnetic resonance in the evaluation of coronary arteries

Sophie Mavrogeni, George Markousis-Mavrogenis, Genovefa Kolovou

Sophie Mavrogeni, George Markousis-Mavrogenis, Genovefa Kolovou, Onassis Cardiac Surgery Center, 17561 Athens, Greece

Author contributions: All the authors both contributed to this paper.

Correspondence to: Sophie Mavrogeni, MD, FESC, Onassis Cardiac Surgery Center, 50 Esperou Street, 17561 Athens, Greece. soma13@otenet.gr

Telephone: +30-210-9882797 Fax: +30-210-9882797

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Abstract

Cardiovascular magnetic resonance (CMR) allows the nonradiating assessment of coronary arteries; to achieve better image quality cardiorespiratory artefacts should be corrected. Coronary MRA (CMRA) at the moment is indicated only for the detection of abnormal coronary origin, coronary artery ectasia and/or aneurysms (class I indication) and coronary bypass grafts (class II indication). CMRA utilisation for coronary artery disease is not yet part of clinical routine. However, the lack of radiation is of special value for the coronary artery evaluation in children and women. CMRA can assess the proximal part of coronary arteries in almost all cases. The best results have been observed in the evaluation of the left anterior descending and the right coronary artery, while the left circumflex, which is located far away from the coil elements, is frequently imaged with reduced quality, compared to the other two. Different studies detected an increase in wall thickness of the coronaries in patients with type I diabetes and abnormal renal function. Additionally, the non-contrast enhanced T1-weighted images detected the presence of thrombus in acute myocardial infarction. New techniques using delayed gadolinium enhanced imaging promise the direct visualization of inflamed plaques in the coronary arteries. The major advantage of CMR

is the potential of an integrated protocol offering assessment of coronary artery anatomy, cardiac function, inflammation and stress perfusion-fibrosis in the same study, providing an individualized clinical profile of patients with heart disease.

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Key words: Coronary angiography; Coronary venous system; Gadolinium; Magnetic resonance imaging

Core tip: Cardiovascular magnetic resonance (CMR) allows the non-radiating assessment of coronary arteries. At the moment it is indicated only to detection of abnormal coronary artery origin, ectasia and/or aneurysms (class I indication) and coronary artery bypass grafts (class II indication). The utilisation of coronary MRA (CMRA) for coronary artery disease diagnosis is not at the moment part of clinical routine. However, due to lack of radiation is particularly useful for children and women. A combined CMR protocol, including CMRA and stress perfusion-fibrosis evaluation may offer a non-invasive assessment of cardiovascular profile in high risk patients.

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INTRODUCTION

Coronary artery disease (CAD) with its sequelae including myocardial infarction and heart failure, is the main cause of increased mortality in our days^[1,2]. The usual way for CAD assessment is the use of invasive coronary angiography; however, the high incidence of CAD and

the queries of invasive assessment necessitate the use of a noninvasive evaluation of coronaries^[3,4].

Cardiovascular magnetic resonance (CMR) can provide a combined approach including coronary arteries, cardiac function and stress myocardial perfusion-fibrosis evaluation. Coronary magnetic resonance angiography (CMRA) has been already used for assessment of coronary anatomy and vessels' wall, providing useful information in CAD^[5-7].

In this review we provide an update of clinical applications of CMRA, discussing the current limitations and the challenges for future applications.

INDICATIONS FOR CMRA

The clinical indications of CMRA are at the moment limited only to the detection of abnormal origin of coronary arteries, coronary ectasia and/or aneurysms (class I indication) and coronary bypass grafts (CABG) evaluation (class II indication). The routine application of CMRA for diagnosis of CAD is not at the moment part of clinical practice^[8,9].

CORONARY VESSELS ABNORMALITIES AND ANEURYSMS (CLASS I INDICATION)

CMRA assesses precisely the abnormal coronary arteries and the location and dimensions of coronary aneurysms. The larger caliber and the proximal location of the coronary artery aneurysms (CAA) facilitate their imaging. The most important benefit of CMRA is the absence of ionizing radiation, which is of special clinical value for children and women^[8,10]. Clinical entities, characterized by ectatic or aneurysmatic coronaries, include Kawasaki disease, autoimmune vasculitis and coronary artery ectasia^[11,12].

KAWASAKI DISEASE AND OTHER AUTOIMMUNE VASCULITIS

In Kawasaki disease, CMR can diagnose lesions both in acute and chronic phase. During the acute phase, a complete evaluation of the coronary anatomy, left and right ventricular function, myocardial inflammation and myocardial fibrosis either due to inflammatory process or due to myocardial infarction is essential.

The presence of CAA needs serial evaluation for patients' risk stratification. Although transthoracic echocardiography is usually sufficient in young children, the visualization of the coronary arteries becomes progressively more difficult as children grow up. According to previous publications, coronary magnetic resonance, using navigator techniques, has an excellent correlation with X-ray coronary angiography using both Pearson coefficient and Bland-Altman analysis and can be used as a reliable alternative for KD patients^[13,14]. Recently, the application of free-breathing techniques in children with KD using the whole-heart approach detected successfully not only the

abnormalities of coronary lumen, but also the abnormally thickened vessel wall and improved risk stratification and monitoring of therapy^[15]. In parallel with coronary assessment, during the same examination, an evaluation of function and wall motion of both ventricles can be also performed using the standard SSFP sequence^[16]. However, only anatomic evaluation is not sufficient to successfully risk stratify KD patients. Previous studies in patients with atherosclerotic coronary artery disease proved that maybe a severe anatomic lesion could not provoke severe myocardial ischemia and in contrary, a marginal coronary lesion can induce significant myocardial ischemia^[17]. Magnetic resonance (MR) first-pass myocardial perfusion imaging during hyperaemia, due to the vasodilating agent adenosine, demonstrates a high diagnostic performance of MR perfusion imaging for the detection of anatomically defined coronary artery stenoses^[18].

Other autoimmune vasculitis that can potentially develop coronary aneurysms include polyarteritis nodosa, microscopic polyangiitis and Wegener granulomatosis^[19]. In these diseases the application of coronary MRA with simultaneous assessment of myocardial oedema-fibrosis may reveal disease activity and pathophysiology of heart lesion noninvasively and without radiation^[20].

CORONARY ARTERY ECTASIA

Coronary artery ectasia (CAE) represents a form of atherosclerosis, detected in 3%-8% of subjects during X-ray coronary angiography. Sluggish blood flow is produced within the ectatic segments, leading to chest pain in effort and myocardial infarction, independently of the significance of coexisting stenosis. CAE is the dilatation of an artery 1.5 times greater than the normal coronary artery and is assessed in 5% of angiographic and in 0.22%-1.4% of autopsy cases^[21-24]. It may involve the entire vessel or be localized in a specific part of the vessel. If it involves the entire vessel, it is called "ectasia". It is due to atherosclerosis in > 50% of cases. Ectasia coexists with coronary artery disease in the majority of patients. Only 10%-20% of CAE coexist with systemic diseases^[25,26], such as scleroderma^[27,28], Ehlers-Danlos syndrome^[29], different types of antineutrophil cytoplasmic antibody (ANCA)-related vasculitis^[19] (Figure 1A), syphilitic aortitis^[30] and Kawasaki disease^[14] (Figure 1B). In some patients, CAE has a congenital origin^[31]. The differentiation between congenital and acquired coronary aneurysms is rather difficult. Acquired CAE should also be differentiated from aneurysms due to different coronary procedures.

The correct follow up of ectatic vessels demands repeated angiograms and CMRA offers an excellent alternative for the evaluation of the initial part of left main, left anterior descending and right coronary arteries^[32]. CMRA has been already proved a valuable clinical tool for diagnosis of abnormal coronary origin, and is in some cases superior to X-ray coronary angiography; however, it is still under investigation for the assessment of the CAD^[32]. Our group proved that CMRA is equal

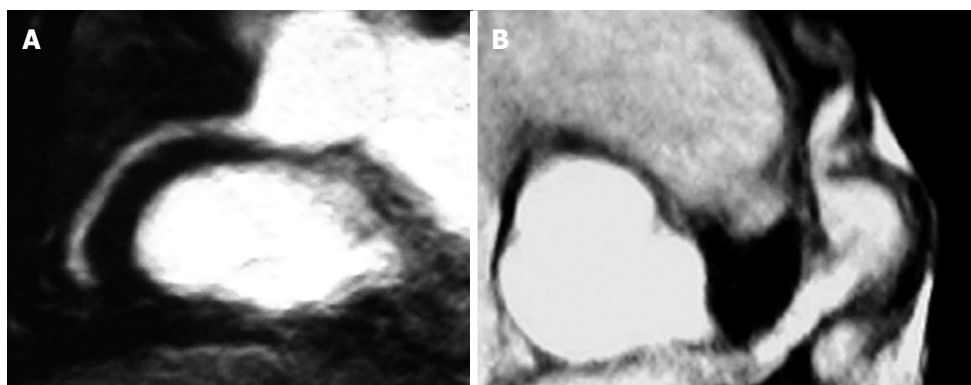


Figure 1 Magnetic resonance angiography. A: Ectatic coronaries in a patient with polyarteritis nodosa, assessed by MRA; B: Aneurysmatic coronaries in a patient with Kawasaki disease, assessed by MRA. MRA: Magnetic resonance angiography.

to quantitative coronary angiography for evaluation of ectatic/aneurysmatic disease. Furthermore, it is a non-invasive, nonradiating technique^[4]. Compared with CT, CMRA does not need use of a contrast agent. CMRA can also give additional data about vessels' blood flow and stress perfusion-fibrosis pattern^[33].

CORONARY BYPASS-GRAFTS (CLASS II INDICATION)

Bypass grafts can be assessed very well by coronary MRA, because they are relatively immobile and have larger diameter compared to coronary arteries. Different imaging ways have been already used, including spin echo^[34-37] and gradient echo techniques. The application of contrast agents for better imaging of the blood signal^[38,39] increased the sensitivity to 95%.

However, metallic clips in grafts constitute the commonest limitation of coronary bypass MRA. Coronary MRA can be used at some special centers to detect lesions in bypass grafts^[8].

CORONARY MAGNETIC RESONANCE ANGIOGRAPHY FOR ASSESSMENT OF CAD

Coronary MRA assesses the initial part of the coronary arteries in almost 100% of patients, with excellent results acquired for the left anterior descending (LAD) and the right coronary artery (RCA); the left circumflex (LCX), due to its peculiar way, is at a increased distance from the cardiac coil, and therefore its visualization is of inferior quality. According to previous studies, the imaged length for LAD is 50 mm, for RCA is 80 mm and for LCX is 40 mm^[40-47]. An excellent agreement between the proximal parts of coronary arteries measured by MRA and by invasive angiography was assessed by previous studies^[48].

Unfortunately, the resolution of CMRA remains lower compared with invasive coronary angiography and does not allow the evaluation of stenosis in small coronary arteries. This is the reason of the low specificity

documented in a recent international multicenter study^[4]; however, CMRA was shown to have a high sensitivity (92%) for the detection of CAD and its diagnostic performance was ameliorated. In a subanalysis of left main or three vessel disease, a sensitivity of 100% and a negative predictive value of 100% was documented. These findings were also supported by smaller single-center studies^[40,49-57].

Recently, a meta-analysis compared coronary MRA and multi-slice computed tomography (CT) for assessment of significant CAD^[34]. CT was more accurate than MRA and therefore CT was suggested as the preferred non-invasive alternative to X-ray coronary angiography. However, the superiority of CMRA is that it can offer more data about the patient, including cardiac anatomy, function, inflammation, stress perfusion and fibrosis evaluation.

Recently, a multicenter study showed that whole-heart CMRA at 1.5 T can detect significant CAD with high sensitivity (88%) and moderate specificity (72%). Additionally, a negative predictive value (NPV) of 88% indicates that this technique can effectively be used to exclude the presence of significant CAD^[58]. We should mention that this NPV reported by this trial is identical to the NPV of the CORE-64 CTA multicenter study^[59]. Proving the value of CMRA to rule out CAD in patients with low pre-test probability (< 20%)^[60].

Finally, in a direct comparison between CMRA and CTA no significant difference was proved for the detection of CAD between 3 T MR and 64-slice CTA^[61]. A comparison between coronary MRA, CTA and invasive coronary angiography (CA) is shown in Table 1.

CORONARY VESSEL WALL ASSESSMENT

The initial CMR images of the coronary vessel wall were taken using fast spin echo techniques^[62,63]. A double inversion recovery preparation was used to take black-blood images improving the contrast between blood and vessel wall^[64]. Recently, the double inversion recovery prepulse has been combined with fast gradient echo^[65], spiral^[66]

Table 1 Comparison between invasive coronary coronary angiography, CTA and magnetic resonance angiography

	CA	CTA	MRA
Noninvasive	No	Yes	Yes
Radiation	Yes	Yes	No
Nephrotoxicity	Yes	Yes	No
Accuracy	+++	++	+
Negative predictive value	+++	+++	++
Cost	High	High	High
Calcium detection	±	+	-
Anomalous coronaries	+++	+++	+++
Ectasia/aneurysm	+++	+++	+++
Graft assessment	+++	+++	+++
CAD evaluation	+++	++	+
Plaque evaluation	+++	±	±

CA: Coronary angiography; MRA: Magnetic resonance angiography; CAD: Coronary artery disease; CTA: Computed tomography coronary angiography.

and radial acquisitions^[67].

Various studies documented the capability of vessel wall imaging to detect remodeling of coronary arteries in CAD and increased vessel wall thickness in type I diabetes with abnormal renal function^[68,69]. It was also documented by Jansen *et al*^[70] that non-contrast enhanced T1-weighted MR visualized thrombus in acute myocardial infarction.

Recently, new techniques using delayed gadolinium enhancement facilitated the direct assessment of inflamed plaques in the coronary arteries. Clinically used contrast agents showed non-specific uptake in plaques of patients with chronic angina^[71]. Acute coronary syndromes^[72] and systemic lupus erythematosus^[73]. The contrast enhancement by CMR, assessed in patients with stable angina, was associated with calcified or mixed plaques on MSCT, while in ACS it was transient, probably due to inflammatory process.

New contrast agents have been already used in animals and their accumulation in blood was associated with increased endothelial permeability and/or increased neo-vascularization^[74]. Additionally, increased accumulation of iron-oxide particles (USPIO) was indicative of increased endothelial permeability and vessel wall inflammation, due to intraplaque macrophages^[75,76].

Such molecules have been used as targets for new molecular contrast agents that allowed the assessment of inflammatory indexes, such as intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1) or matrix metalloproteinase (MMP)^[77,78]. Furthermore, thrombi labeling using a fibrin-specific contrast agent^[79,80] and evaluation of extracellular matrix remodeling, using targeting elastin is a new promising molecular imaging technique^[81,82] for early detection of plaque vulnerability^[83].

CONCLUSION

CMR is a non-invasive, non-radiating technique for evaluation of coronary arteries and coronary wall. Its

major advantage is the potential of a combined protocol, including coronary arteries, cardiac anatomy, function, inflammation and stress perfusion-fibrosis in the same study in CAD and/or heart failure.

CMRA current indications include: (1) assessment of abnormal coronary arteries, coronary ectasia and/or aneurysm (class I indication); and (2) coronary bypass grafts (class II indication). In the future, it may be used to exclude CAD in selected patients. However, further improvements are needed to support its use for routine assessment of high risk populations.

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WJC 6th Anniversary Special Issues (5): Myocardial infarction

ST-segment elevation: Distinguishing ST elevation myocardial infarction from ST elevation secondary to nonischemic etiologies

Alok Deshpande, Yochai Birnbaum

Alok Deshpande, Yochai Birnbaum, The Section of Cardiology, The Department of Medicine, Baylor College of Medicine, Houston, TX 77030, United States

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Supported by John S Dunn Chair in Cardiology Research and Education

Correspondence to: Yochai Birnbaum, MD, John S Dunn Chair in Cardiology Research and Education, The Section of Cardiology, The Department of Medicine, Baylor College of Medicine, One Baylor Plaza, MS: BCM 620, Houston, TX 77030, United States. ybirnbau@bcm.edu

Telephone: +1-713-7982735 Fax: +1-713-7980270

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Abstract

The benefits of early perfusion in ST elevation myocardial infarctions (STEMI) are established; however, early perfusion of non-ST elevation myocardial infarctions has not been shown to be beneficial. In addition, ST elevation (STE) caused by conditions other than acute ischemia is common. Non-ischemic STE may be confused as STEMI, but can also mask STEMI on electrocardiogram (ECG). As a result, activating the primary percutaneous coronary intervention (pPCI) protocol often depends on determining which ST elevation patterns reflect transmural infarction due to acute coronary artery thrombosis. Coordination of interpreting the ECG in its clinical context and appropriately activating the pPCI protocol has proved a difficult task in borderline cases. But its importance cannot be ignored, as reflected in the 2013 American College of Cardiology Foundation/American Heart Association guidelines concerning the treatment of ST elevation myocardial infarction. Multiples strategies have been tested and studied, and are currently being further perfected. No matter

the strategy, at the heart of delivering the best care lies rapid and accurate interpretation of the ECG. Here, we present the different patterns of non-ischemic STE and methods of distinguishing between them. In writing this paper, we hope for quicker and better stratification of patients with STE on ECG, which will lead to better outcomes.

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Key words: Diagnosis; Electrocardiogram; Reperfusion therapy; ST segment elevation; Myocardial infarction

Core tip: At times, distinguishing between myocardial infarction with ST elevation (STEMI) from non-ischemic causes of elevation of the ST segment is difficult, especially in patients with atypical presenting symptoms. Understanding common patterns of ST elevation that are not caused by ischemia is crucial for rapid and accurate diagnosis. However, patients with baseline non-ischemic ST elevation (for example, early repolarization or repolarization changes caused by hypertrophy of the left ventricle) may develop acute myocardial infarction (true STEMI or non-ST elevation myocardial infarction with baseline ST elevation). Here we describe common patterns of non-ischemic ST elevation.

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INTRODUCTION

Today, the electrocardiogram (ECG) is the most com-

monly used diagnostic tool for recognizing and triaging of patients with symptoms suggestive of myocardial infarction (MI). Per the “Third Universal Definition of Myocardial Infarction” document, the ECG should be acquired and interpreted within 10 min of presentation^[1]. Additionally, serial ECGs every 15 to 30 min should be performed in patients with ongoing symptoms in whom the initial ECG is not diagnostic of ST elevation MI (STEMI)^[1].

ST elevation (STE) is considered to reflect acute transmural ischemia caused by an occlusion of an epicardial coronary artery by a blood clot. Therefore, it is recommended that patients with suspected acute STEMI and without contraindications should be subjected as soon as possible to therapy intended to recanalize the occluded artery by either primary percutaneous coronary intervention (pPCI) or fibrinolysis. In contrast, the guidelines recommend initial conservative therapy for patients with suspected MI without STE, as active ongoing ischemia may not be present and earlier studies have not shown a benefit for reperfusion therapy in patients without STE^[2].

As per the 2013 ACCF/AHA Guideline for the Management of ST-Elevation Myocardial Infarction, ST elevation myocardial infarction is a clinical syndrome that compromises typical symptoms of acute ischemia of the heart muscle in conjunction with elevation of the ST segment and increased blood levels of biomarkers that indicate necrosis of the cardiac muscle. By these guidelines, pPCI is recommended for those with symptoms indicative of ischemia of the heart muscle that began 12 h or less before medical encounter who have elevation of the ST segment^[3]. Although the innovation of cardiac troponin (cTn) assays specific to the myocardium is changing the overall diagnosis of MI, the decision to proceed with angiography or give thrombolytics is made based on STE on the ECG and is usually reached before troponins are detectable in the blood. Further, the elderly, patients of female gender, and diabetic patients frequently present with symptoms that are not typical, further emphasizing the role of the presenting ECG for diagnosis and triage of such individuals^[4-6].

In most of individuals without prior cardiac disease, the ST segment is at the level of the preceding P-R segment and/or the following T-P segment (so called isoelectric). Deviation of the ST-segment (elevation or depression compared to the isoelectric line) can be a sign of ischemia of the heart muscle. However, deviations of the ST segment relative to the isoelectric line due to nonischemic etiologies are often seen. Elevation of the ST segment due to non-ischemic etiologies was reported up to 15% in the general population. One study^[7] found that 91% of 6014 men who served in the United State Air Force, between 16 and 58 years of age, without any apparent cardiac disease had elevation of the ST segment of 0.1 to 0.3 mV in more than one of the precordial leads (most commonly seen in lead V2). Another study suggested that elevation of the ST segment above 0.1 mV in one or more leads (V1 to V4) in 529 men without apparent cardiac disease could be found in 93% among those who

were between 17 and 24 years of age. As age progresses, the prevalence of elevation of the ST segment declined^[8]. Thus, most men have elevation of the ST segment greater than 0.1 mV in the precordial leads. Therefore, elevation of the ST segment should be regarded as a normal finding and is often termed “male pattern”. On the other hand, only fifth of patients of female gender have elevation of the ST segment above 0.1 mV, and this percentage is not influenced by the age of the female patients^[9]. These thresholds are discussed in the “Third Universal Definition of Myocardial Infarction” document^[1].

Different cutoffs for the amount of STE are causing confusion. The cutoffs for abnormal elevation of the ST segment, per the “Third Universal Definition of Myocardial Infarction” document for leads V2-V3, are elevation of the ST segment at the J-point of above 0.2 mV in men 40 years of age or older, 0.25 mV or above in men below 40 years of age, and 0.15 mV or above in women and/or 0.1 mV or above in all other leads in patients without hypertrophy of the left ventricle or block of the left bundle branch^[1]. These criteria are based on the 2% extreme outside of the mean calculated from a population of 1321 Caucasians from the city of Glasgow and the region of Strathclyde in Scotland^[9]. The 2013 ACCF/AHA STEMI guidelines have simplified these recommendations. In these guidelines STE at the J point in 2 contiguous leads or more of 0.2 mV or more in males or 0.15 mV or more in women in leads V2-V3 and/or of 0.1 mV or more in all other leads is the threshold^[3]. Considering the ethnic homogeneity and the decreasing STE magnitude with age, these cutoffs should be appreciated in this context^[9]. It is unclear whether the same thresholds for STE can be used in populations of different ethnicity, as higher magnitude of STE was reported in Nigerian healthy men^[10]. It is plausible that if the thresholds, endorsed by the “Third Universal Definition of Myocardial Infarction” document are used, the reported incidence of anterior STEMI would decrease, especially in men younger than 40 years of age. Moreover, currently there are no guidelines as to what are considered “normal” STE for patients whose ECG shows criteria for hypertrophy of the left ventricular, left bundle branch block or other forms of advanced intraventricular conduction defects.

As abovementioned, many patients presenting with typical symptoms have elevation of the ST segment due to non-ischemic etiologies (NISTE)^[3-6]. Physicians must use all tools at their disposal to reach accurate diagnosis and reduce the risk of false activation of the pPCI protocol or exposure to thrombolytic therapy from one hand, while not missing cases of true STEMI. There are patterns of NISTE that are frequent and typical and can be easily recognized and distinguished from ST elevation myocardial infarction. Yet, there are individuals with pre-existing ST elevation secondary to non-ischemic etiologies (*e.g.*, hypertrophy of the left ventricle or “early repolarization”) that can develop superimposed acute MI (ST elevation myocardial infarction or non-STEMI (NSTEMI)); therefore, presence of benign patterns of NISTE does not always rule out acute coronary syndrome (ACS)

Table 1 Common patterns of nonischemic ST elevation

ST elevation secondary to LVH
ST elevation secondary to conduction defect (such as left bundle branch block and non-specific intracardiac conduction delay)
Early repolarization pattern (notched J-point typically in anterolateral leads)
Normal variant of ST elevation (ST elevation mostly in leads V2-V3)
Concave ST elevation
Spontaneously reperfused STEMI
Aneurysm/old myocardial infarction
Pericarditis/myocarditis
Wolf-Parkinson-White syndrome (pre-excitation)
Brugada pattern
Takotsubo (apical ballooning) syndrome
Hyperkalemia
Hypercalcemia

LVH: Left ventricular hypertrophy; STEMI: ST elevation myocardial infarctions.

and even STEMI.

The differential diagnosis of elevation of the ST segment is wide, including conditions with secondary ischemia of the myocardium (for example, dissection of the aortic wall), pre-existing elevation of the ST segment without acute ischemia, and instances with new elevation of the ST segment with chest pain but without evidence of ischemia of the heart muscle (for example, myocarditis or pericarditis, pulmonary embolus, electrolyte imbalance, rate-related repolarization changes, *etc.*). Obviously, with the current emphasis on diagnosing and triaging acute ST elevation myocardial infarction rapidly, the probability of over-diagnosing ST elevation myocardial infarction and false activation of the pPCI protocols or administration of fibrinolytic therapy may increase.

Failure to identify NISTE has its costs. It may delay treatment for the original medical condition (*i.e.*, aortic dissection, pulmonary embolus, peptic disease, *etc.*) and may expose the patient to unnecessary irradiation and exposure to contrast agents, in addition to increased health care costs and exhaustion of the catheterization laboratory personnel.

False-positive activation of the catheterization laboratory (no culprit lesion) have been reported in 9% to 14% of the patients^[11,12]. More importantly, inappropriate activation rate, where the cardiologist did not perform an emergent coronary angiogram, is varied from 5% to 23%^[12], largely depending on the training of the activator (paramedic or ED physician).

In this paper, we describe different patterns of STE and their underlying causes. We intend to provide insight into pathological *vs* non-pathological STE (Table 1). A better understanding of STE will lead to faster and the more appropriate treatment, lower false-positive and inappropriate activation of urgent reperfusion protocols (fibrinolytic therapy or pPCI), ensuring the best patient outcomes.

“CONVEX” VS “CONCAVE” PATTERNS OF STE

As mentioned above, the ST segment is normally isoelec-

tric. ST elevation with convex or straight pattern is traditionally considered as indicative of STEMI in contrast to a concave pattern, which is typically considered to be secondary to nonischemic etiologies. The 2004 ACCF/AHA guidelines supported this belief^[13]; however, this recommendation has been omitted from the current 2013 ACCF/AHA guidelines^[1].

Wang *et al*^[14] also emphasized the importance of the concave tracing in establishing the male pattern, left bundle branch block (LBBB), and LVH forms of STE over STE-MI. However, concavity versus convexity must be analyzed carefully and should not be relied on as the sole criteria for distinguishing NISTE from STEMI. Brady *et al*^[15] reported 77% sensitivity, 97% specificity, 94% positive predictive value, and 88% negative predictive value for a non-concave STE morphology in acute MI diagnosis. Given the use of ECG for screening, such suboptimal sensitivity would yield poor patient outcomes. Figure 1 depicts an ECG of a man with acute anterior wall ST elevation myocardial infarction presenting with concave form of ST elevation in the precordial leads. Angiography of the coronary arteries revealed total occlusion of the left anterior descending coronary artery (LAD) and the STE resolved after pPCI.

EARLY REPOLARIZATION

The “early repolarization” pattern is usually found 1% to 5% of the population. Most commonly found in young, athletic, black males^[16,17]. In the past, early repolarization pattern of NISTE was considered a benign pattern^[17]. More recently, however, early repolarization pattern has been associated with cardiac arrhythmia and sudden cardiac mortality, mainly if there is 0.2 mV or more elevation of the ST segment. Nevertheless, this pattern is not caused by acute ischemia mandating emergent reperfusion therapy. The typical pattern appears as no S wave in V₃; 1-4 mm concave elevation of the ST-segment in leads V2-V5 (most prominent in V3) and sometimes the inferior leads; and notching of the downstroke of the R waves (“J” wave), most distinct in lead V5 and V6^[16-18]. However, other authors have used different definitions. Figure 2 is an example of early repolarization pattern.

In many cases of “early repolarization”, elevation of the ST segment is not lasting and decreases or disappears when the heart rate increases or if the patient hyperventilates. Therefore, significant changes in the magnitude of ST elevation are not necessarily diagnostic for acute myocardial ischemia. At times, concomitant inversion of the T-waves may be present in the precordial leads, which are due to “juvenile T wave pattern” in younger subjects. These changes could be mistaken for acute myocardial ischemia^[16].

Hypothermia may cause prominent J-point notch (Osborne waves)^[19] that must be distinguished from “early repolarization” pattern. Hypothermia frequently causes slow heart rate and muscle shiver. Osborne waves with elevation of the ST segment are occasionally seen in patients with severe hypercalcemia or disorders of the central nervous system. Low body temperature usually



Figure 1 A patient with acute anterior wall ST elevation myocardial infarctions with concave form of ST elevation in the precordial leads (V3-V5). Coronary angiography revealed mid left anterior descending occlusion and primary percutaneous coronary intervention was performed.

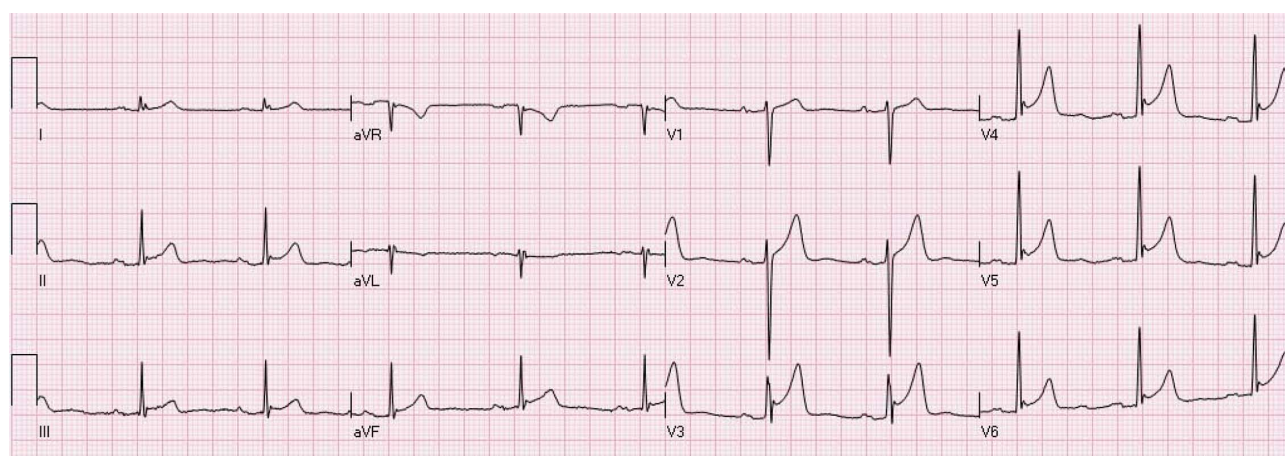


Figure 2 An example of ST elevation due to “early repolarization”. ST elevation with notched J waves is seen in the inferior and anterolateral leads.

causes prolongation of the QT interval. On the other hand, hypercalcemia usually induces shortening of the QT interval^[20]. Hyperkalemia can also cause elevation of the ST segment. In addition, hyperkalemia often presents with QRS widening and changes can be seen in the P waves and the PR segments. Another entity that can be mistaken for notching of the J-point (so called “epsilon waves”) is typically observed in “Arrhythmogenic Right Ventricular Dysplasia”. In arrhythmogenic right ventricular dysplasia, however, epsilon waves are commonly present in the precordial leads V1-V3^[21].

A “NORMAL-VARIANT” PATTERN OF NISTE

A “normal-variant” ST elevation typically presents as elevation of the ST segments mainly in the precordial leads V1 to V3 (Figure 3)^[14]. It is typically seen in young persons, mainly in Hispanic or African American males. QRS criteria for left ventricular hypertrophy are not met and concomitant depression of the ST segments and T waves changes in the lateral leads are not seen. There are

investigators who do not make the difference between a “normal variant” pattern and “early repolarization” pattern, grouping them together under the “early repolarization” umbrella. It should be remembered that “early repolarization” and “normal variant” patterns are frequently present in the same patients.

ELEVATION OF THE ST SEGMENTS DUE TO HYPERTROPHY OF THE LEFT VENTRICLE

Just as the QRS complex amplitude may increase by a more massive left ventricle, changes in the ST segments can be amplified^[22]. NISTE due to hypertrophy of the left ventricle (LVH) is usually seen in leads V1-V3. Typically, there are QRS amplitude criteria for hypertrophy of the left ventricle and associated depression of the ST segments in the leads facing the lateral wall (V5-V6 and I and aVL) (Figure 4). Frequently elevation of the ST segment is seen in lead aVR. It is crucial not to misdiagnose this pattern as the pattern thought to represent left main

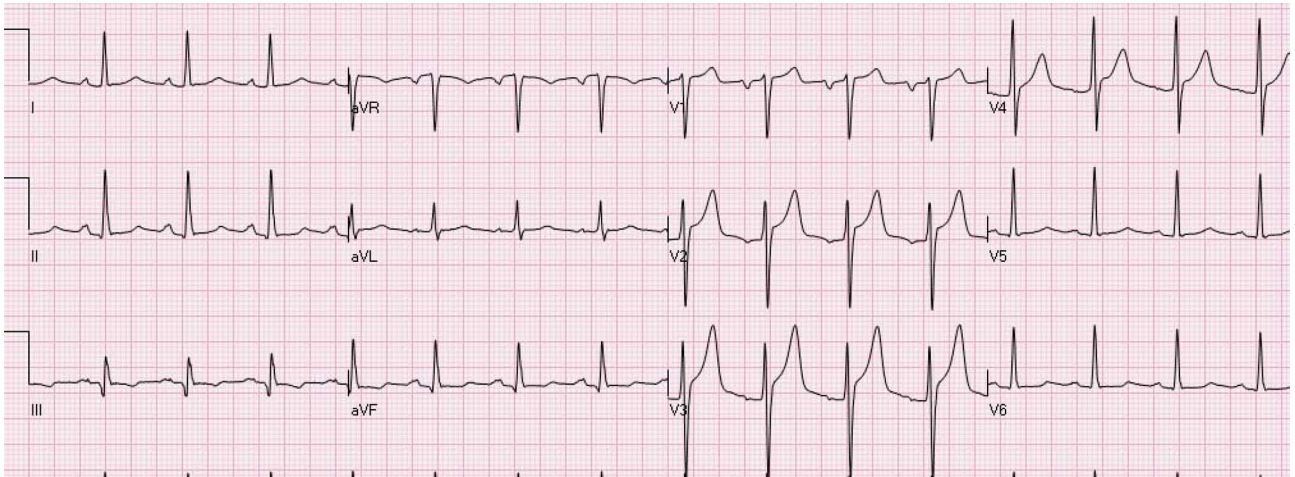


Figure 3 An electrocardiogram of a young male with a “normal variant” concave pattern of ST elevation in leads V2-V4.

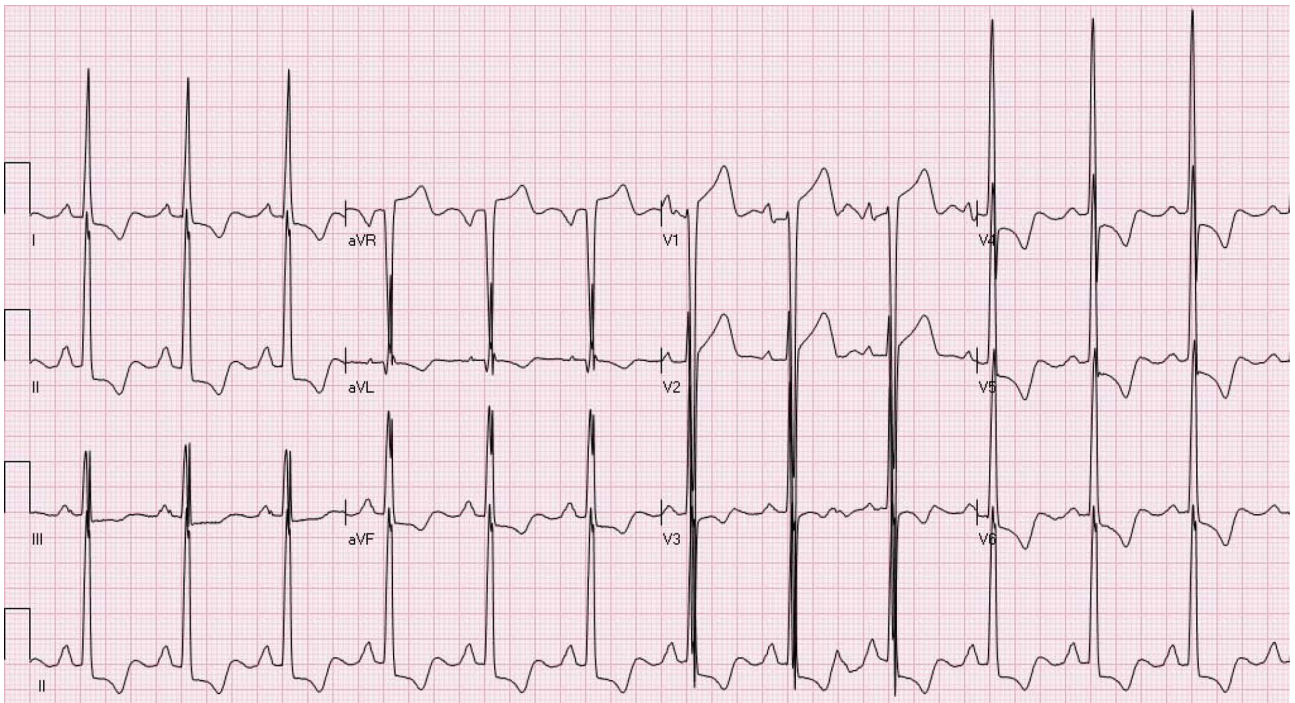


Figure 4 An electrocardiogram showing typical pattern of ST elevation due to hypertrophy of the left ventricular with secondary repolarization changes. There is ST elevation in leads V1-V2 and ST depression with T wave inversion in the inferolateral leads.

induced- or circumferential- subendocardial ischemia (elevation of the ST segments in leads V1 and aVR with accompanying depression of the ST segments in the inferior as well as the anterolateral leads). Per the Third Global MI Task Force consensus paper^[1], the cutoffs for the absolute amplitude of the ST segment elevation do not apply for patients with hypertrophy of the left ventricle. Yet; hypertension is an established risk factor for atherosclerotic heart disease, including acute MI. It should be remembered, however, that at times hypertrophy of the left ventricle may present with atypical configurations of ST elevation (Figure 5). Furthermore, frequently patients are presenting with more than one pattern of NISTE (LVH + early repolarization or nonspecific intraventricu-

lar conduction delay [IVCD] + LVH and even STEMI on top of ST segment deviations induced by LVH).

ACUTE PERICARDITIS

STE may be seen in the acute or first stage of pericarditis, which occurs in the first few days and may last up to weeks. In most cases, diffuse STE is seen in all the ECG leads, except in leads aVR and V1, that typically have reciprocal depression of the ST segments (Figure 6). This pattern is often associated with PR depression in all ECG leads, except leads V1 and aVR, which occasionally depict reciprocal PR elevation^[23]. Focal pericarditis (for example, after acute myocardial infarction or heart surgery), how-

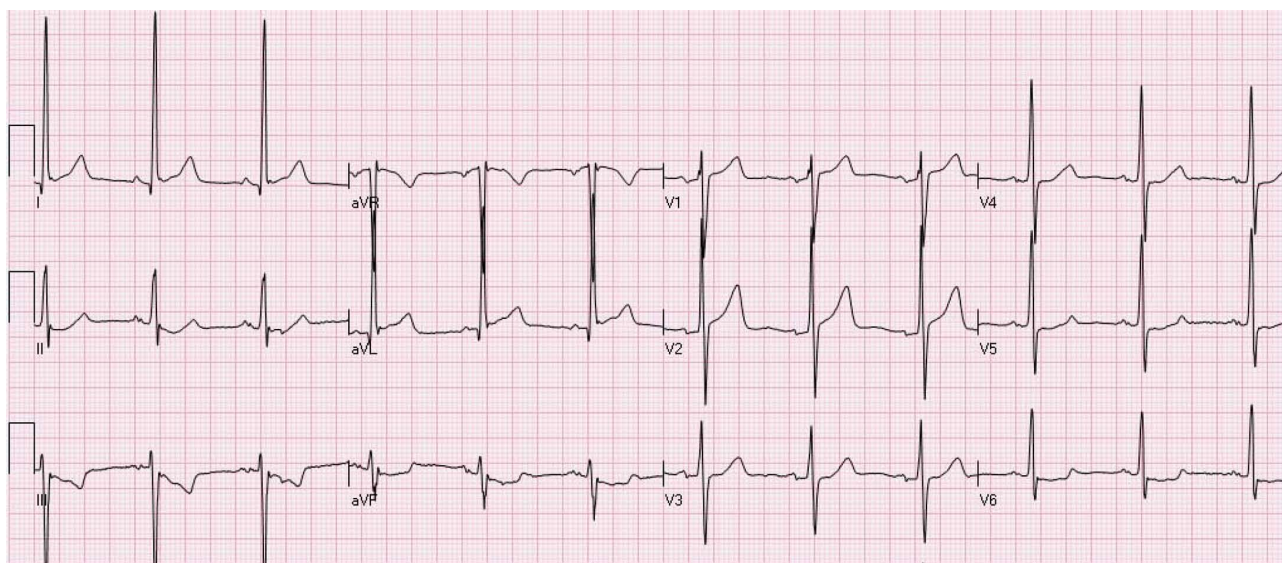


Figure 5 An electrocardiogram of a patient with atypical form of ST elevation secondary to left ventricular hypertrophy. ST elevation is present in leads I, aVL, V1-V2. Mild ST depression is present in the inferior leads and V5-V6.

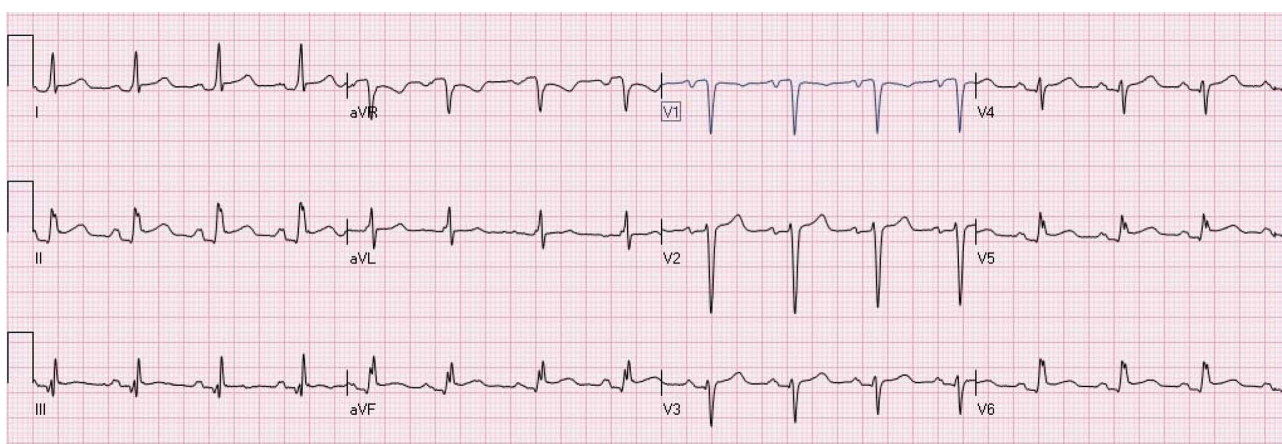


Figure 6 Diffuse ST elevation secondary to acute pericarditis. There is typical depression of the PR segment (seen mainly in leads II and aVF). There is ST elevation in the inferolateral leads with ST depression in lead aVR.

ever, may induce more regional and non-typical forms of STE, which at times could be associated with depression of the ST segments in leads other than V1 and aVR. These atypical patterns could be mistaken for STEMI.

STE SECONDARY TO LEFT BUNDLE BRANCH BLOCK

LBBB typically causes marked ST changes (Figure 7), making it difficult to recognize STEMI when the LBBB pattern is present.

New or presumably new LBBB was regarded in the past as an STEMI equivalent^[13]. However, the majority of cases with LBBB at the time of presentation, are “not known to be old”, simply because an ECG prior to the index presentation is not available for comparison. Presumably new LBBB and even new LBBB at presentation occurs infrequently, is interfering with the analysis of the

ECG, and according to the current STEMI guidelines are not considered diagnostic of acute myocardial infarction without the presence of typical clinical symptoms^[3].

Only 1% to 9% of patients suspected of an acute myocardial infarction have LBBB (new or old) on their ECG^[24]. Of the patients with LBBB on whom the STEMI protocol was initiated, 39% had a final diagnosis of true ACS, 36% had cardiac diagnoses other than ACS (hypertensive emergency, acute heart failure, atrial fibrillation, complete heart block, severe aortic stenosis, *etc.*) and 25% had non-cardiac chest pain^[25,26].

LBBB pattern inherently has a masking feature that hides STEMI. ST deviation typically is directed opposite to the direction of the QRS complex. Acute STEMI, on the other hand, typically presents with ST segment deviations that are concordant with the QRS complex deflections. As patients with LBBB typically show negative QRS complexes in leads V1- V3 (deep S waves), they typically have elevations of the ST segment in the precordial leads V1-V3.

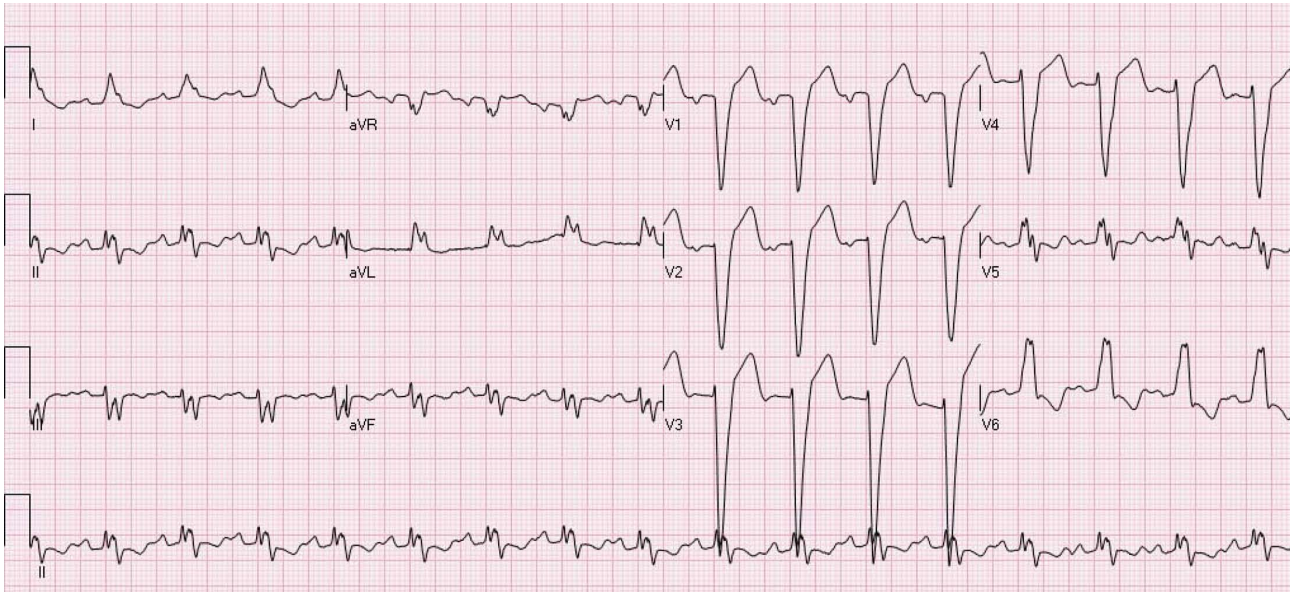


Figure 7 ST elevation secondary to left bundle branch block. The ST segment vector is directed opposite to the QRS vector. STE is present in the leads with negative QRS deflection (mainly leads V1-V3). There is typical ST depression in the leads with positive QRS deflection (the inferolateral leads).

This pattern must not be confused with anterior STEMI.

Criteria for recognizing STEMI in patients with LBBB were published by Sgarbossa and colleagues: (1) STE more than 0.1 mV that is concordant with the vector of the QRS complex; (2) ST depression of more than 0.1 mV in lead V1, V2, or V3; and (3) STE of more than 0.5 mV that is directed opposite to the QRS direction^[27,28]. Patients are given a point for each of the above criteria and can be stratified on the likelihood of having STEMI based on their Sgarbossa score.

These criteria have been validated in multiple studies^[24]. These criteria are reported to have high specificity; however, their sensitivity for identifying acute myocardial infarction in patients presenting with LBBB is low^[24,29]. In a recent meta-analysis, a three point Sgarbossa criteria score (≥ 0.1 mV of concordant STE or ≥ 0.1 mV ST depression in leads V1 to V3) had a sensitivity of 20% and specificity of 98%. If the third original criterion of discordant STE ≥ 0.5 mV in leads is added, the reported sensitivity is ranging between 20% and 79% and specificity between 61% and 100%^[29].

Smith and colleagues^[30] suggest replacing the third criteria of > 5 mm absolute deviation in leads with discordant QRS complex with an ST/S ratio ≤ -0.25 . Doing so increased the sensitivity from 67% to 91%, but the specificity remained unchanged at 90%. This modified Sgarbossa criteria needs to be validated with further studies^[30].

The absolute magnitude of the deviation of the ST segments in patients with LBBB is influence by the degree of aberrancy and could change secondary to changes in the QRS axis, duration or heart rate. In addition, the absolute magnitude of deviation of the ST segment could change between different ECGs secondary to different electrode placement; this is often observed in the anterolateral precordial leads (V4-V6) in patients showing axis deviation to the left.

There is no data as to the thresholds of ST segment deviation in cases with incomplete LBBB (iLBBB; QRS duration of < 120 msec). Especially, it is unclear what are the cutoff values of “normal” STE in the precordial leads V1-V3 in cases with iLBBB.

STE SECONDARY TO OTHER INTRAVENTRICULAR CONDUCTION DELAYS

Patients with nonspecific intraventricular conduction delay may also display ST changes secondary to repolarization abnormalities (Figure 8). The pattern and magnitude of ST segment elevation or depression in such patients is highly variable, and the right diagnosis of STEMI can often be made only with comparison the index ECG and previous tracings or following changes over time in followup ECGs. Once more, the absolute magnitude of ST deviation can change as the degree of conduction delay changes (QRS width and axis) and may also depend on the heart rate.

Right bundle branch block (RBBB) is considered not to affect the interpretation of ST elevation or depression. Tachycardia, however, may cause depression of the ST segments in the right precordial leads (V1-V3) in patients with RBBB. These dynamic changes in the ST segments are often mistakenly diagnosed as true inferolateral (posterior) STEMI equivalent.

Pre-excitation (Wolf-Parkinson-White pattern) is occasionally associated with NISTE that are secondary repolarization alterations. The absolute magnitude of ST segment deviation is highly affected by the degree of pre-excitation.

BRUGADA SYNDROME

The Brugada pattern includes a pattern resembling RBBB

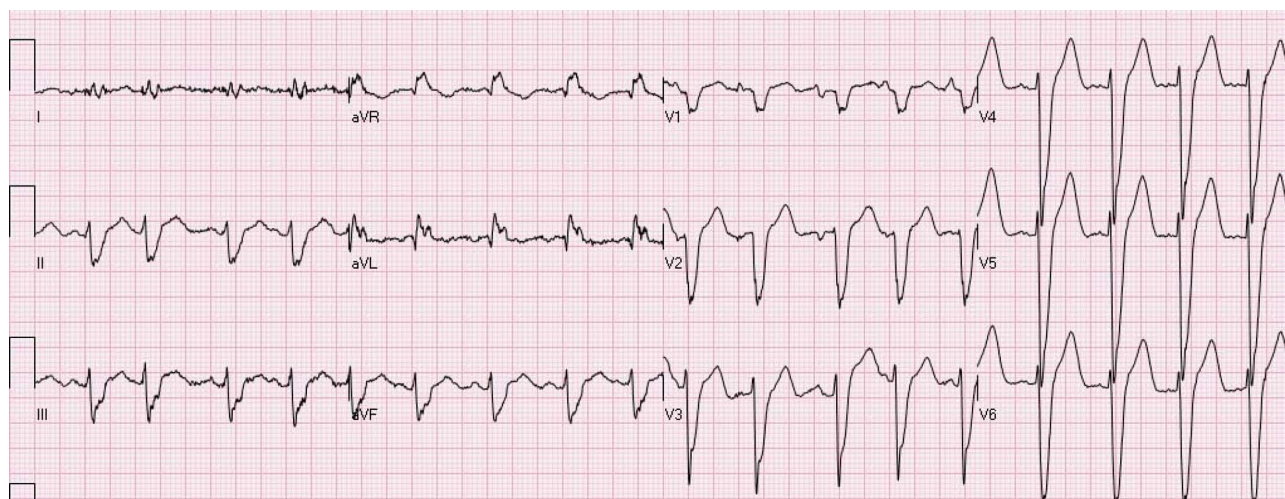


Figure 8 A patient with intraventricular conduction delay. There is mild elevation of the ST segment in the inferior leads and marked ST elevation in V2-V6. The patient is known to have non-ischemic dilated cardiomyopathy and this is his chronic electrocardiogram pattern.



Figure 9 A patient showing a Brugada pattern with RsR', elevation of the ST segment and negative T waves in leads V1-V2.

with elevation of the ST segment in the precordial leads (V1-V2)^[31,32]. The Brugada syndrome is linked to an increased risk of ventricular arrhythmia and sudden cardiac death. Type 1 Brugada pattern is defined by a coved elevation of the ST segment more than 0.2 mV, associated T wave inversion in more than one of the right precordial leads (V1-V3). This pattern can be seen spontaneously or only after the administration of a sodium channel blocker. The diagnosis of Brugada syndrome depends on the presence of ECG Brugada pattern in a patient with documented history of ventricular fibrillation or polymorphic ventricular tachycardia, or a history of sudden cardiac death in family members that are younger than 45 years, comparable ECG configuration in relatives, unexplained syncope, ability to induce of ventricular tachycardia with programmed electrical stimulation, or agonal respiration at night time^[33]. Type 2 Brugada pattern typically presents with a saddleback pattern of STE of more than 0.2 mV that attenuates in the middle and distal part of the ST segment with a positive or biphasic T waves in the precordial leads V1 to V3. Type 3 Brugada pattern shows

a saddleback or coved pattern of elevation of the ST segment (less than 0.1 mV). Type 2 and 3 Brugada patterns should not be used to diagnose the Brugada syndrome and are not associated with increased risk of ventricular arrhythmia and sudden death. The ECG changes associated with the Brugada syndrome fluctuate with time, with diverse patterns and magnitude of elevation of the ST segments seen on different ECG tracings^[33]. Figure 9 is an example of type 1 Brugada pattern.

TAKOTSUBO SYNDROME (APICAL BALLOONING SYNDROME)

Takotsubo syndrome is seen mainly in females after menopause. Typically, the syndrome follows acute physiologic or emotional stress. The subjects frequently experience chest pain or dyspnea. The presenting ECG typically shows elevation of the ST segment in the majority (up to 81.6%) of the patients. STE is typically seen in the precordial leads. In addition, abnormalities of the T waves (64.3%) and Q waves (31.8%) can be detected. Takot-

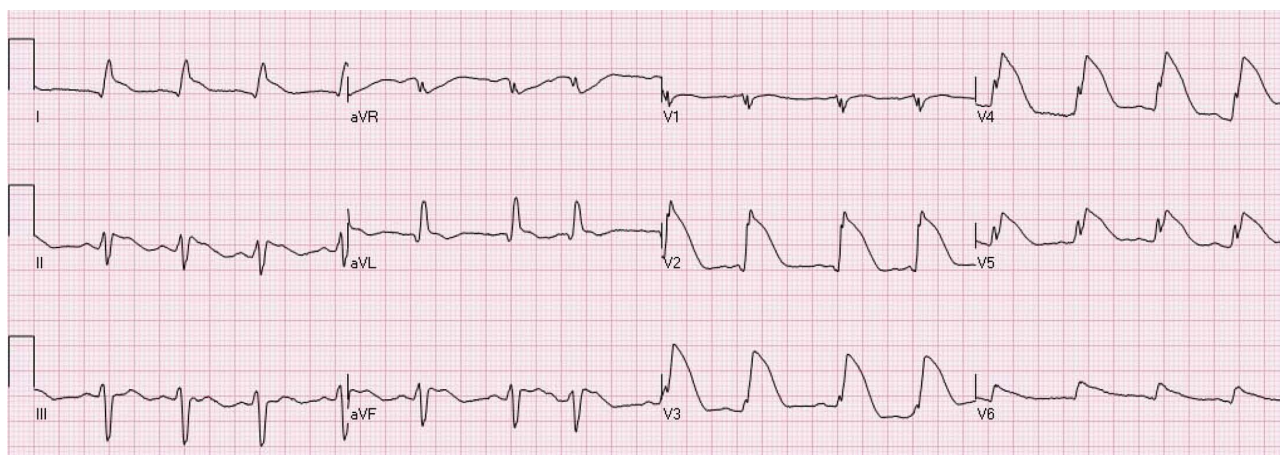


Figure 10 An elderly female patient with ST elevation secondary to Takotsubo. Angiography of the coronary arteries did not demonstrate significant coronary artery narrowings.

subo syndrome is typically associated with mild elevation of the cardiac markers (up to 86.2% of the patients)^[34].

In the acute phase, the ECG of Takotsubo patients classically show marked depression of the ST segment in lead aVR, no or minimal elevation of the ST segment in lead V1 and concomitant diffuse STE in the other ECG leads (Figure 10)^[35].

Frequently, the initial ECG pattern is mistaken for anterior STEMI (especially the type caused by distal occlusion of a wrapping long LAD that results in concomitant STE in both the inferior and precordial leads). Echocardiogram may show regional wall motion abnormalities that are confined to the apex. This pattern is not typical of the common type of anterior STEMI, but may be seen in patients with apical occlusion of a wrapping LAD. It was suggested that the ECGs of patients with Takotsubo have more marked depression of the ST segment in lead aVR in conjunction with less elevation of the ST segment in the precordial lead V1 relative to the ECGs of patients with typical acute anterior STEMI^[36].

Because of the variations in presentation, Takotsubo's may be confused for other STE causes as well. Since many patients present with PR depression, Takotsubo cardiomyopathy often resembles acute pericarditis on EKG^[35].

Acute stroke (particularly subarachnoid hemorrhage)^[37,38] and pheochromocytoma^[39,40] occasionally present with ECG and regional wall motion dysfunction findings that are indistinguishable from Takotsubo cardiomyopathy.

SPONTANEOUSLY REPERFUSED STEMI

The current STEMI guidelines advocate that subjects presenting with symptoms suggestive of ACS within the 12 h before presentation who have elevation of the ST segment in 2 or more adjacent ECG leads should undergo reperfusion therapy as soon as possible^[3]. However, a significant percentage of patients probably have (partial) decrease in the severity of symptoms by the time of arrival to the hospital, more often after receiving chewable aspirin on route to the hospital. In many patients with

spontaneous reperfusion the ECG depicts (incomplete) decline in STE with concomitant inversion of the last part of the T waves, as shown in Figure 11. This entity is not recognized by the current guidelines and there are no recommendations whether coronary angiography and revascularization can be delayed in patients with clinical suspicion of "spontaneous reperfusion" if they present within 12 h of onset of symptoms and still have some degree of STE. On the other hand, urgent pPCI is not recommended to asymptomatic patients who are hemodynamically stable despite having STE, if they present > 12 h of onset of symptoms^[3].

LEFT VENTRICULAR ANEURYSM

Left ventricular aneurysm may result in persistent elevation of the ST segment after a previous MI. Frequently, the ECG may be very similar to that of acute STEMI. In fact, STE secondary to aneurysm may be the most frequently misinterpreted pattern in patients presenting to the emergency room with pain in their chest or dyspnea^[41]. In Brady and colleagues' study, where 11 hypothetical patients and accompanying EKGs were presented to 458 Emergency room physicians, left ventricular aneurysm was misdiagnosed 72% of the time, making it the most commonly misinterpreted STE pattern^[42]. Diagnosis is extremely difficult when previous ECGs are unavailable for comparison. Typically, the ECGs of patients with left ventricular aneurysm depict abnormal Q waves in the ECG leads showing elevation of the ST segment. Figure 12 is an example of persistent STE due to aneurysm in a patient three months after acute MI.

MIXED PATTERNS

In a large number of patients the ECG may show more than one pattern of elevation of the ST segments that makes the precise distinction between NISTE and STEMI extremely hard. At times patients with preexisting benign pattern of NISTE may present with chest pain secondary to NSTEMI. This is termed "pseudo"-STEMI

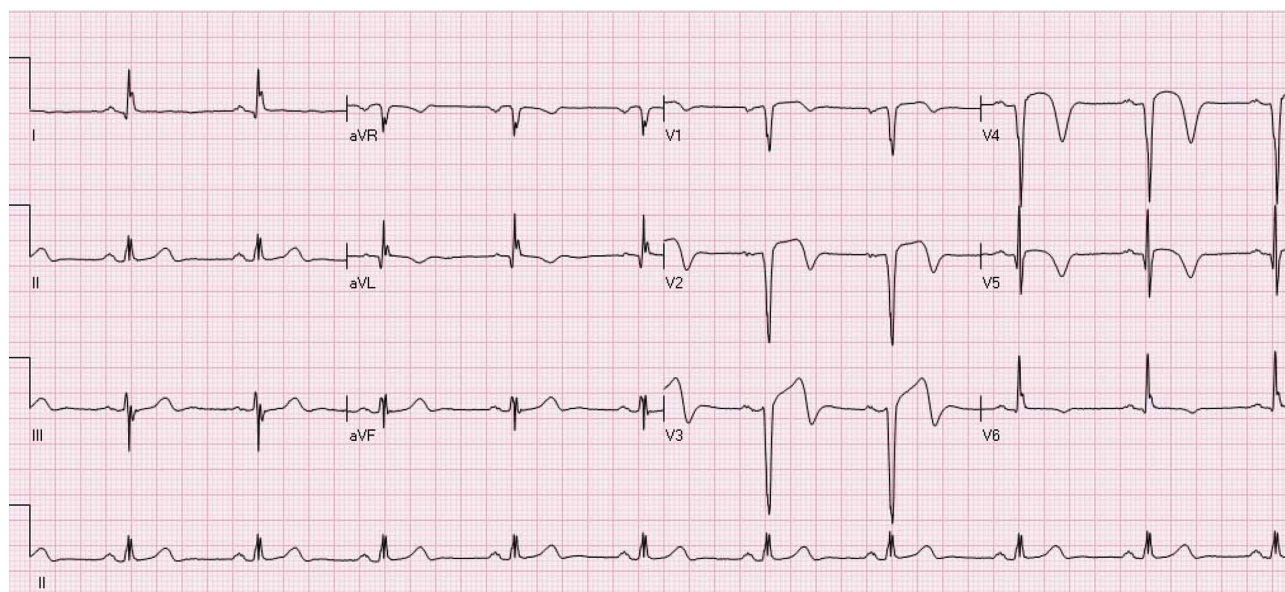


Figure 11 A patient with ST elevation associated with inversion of the terminal portion of the T waves in leads aVL, V1-V5 due to recent anterior ST elevation myocardial infarction. On presentation symptoms have already subsided.

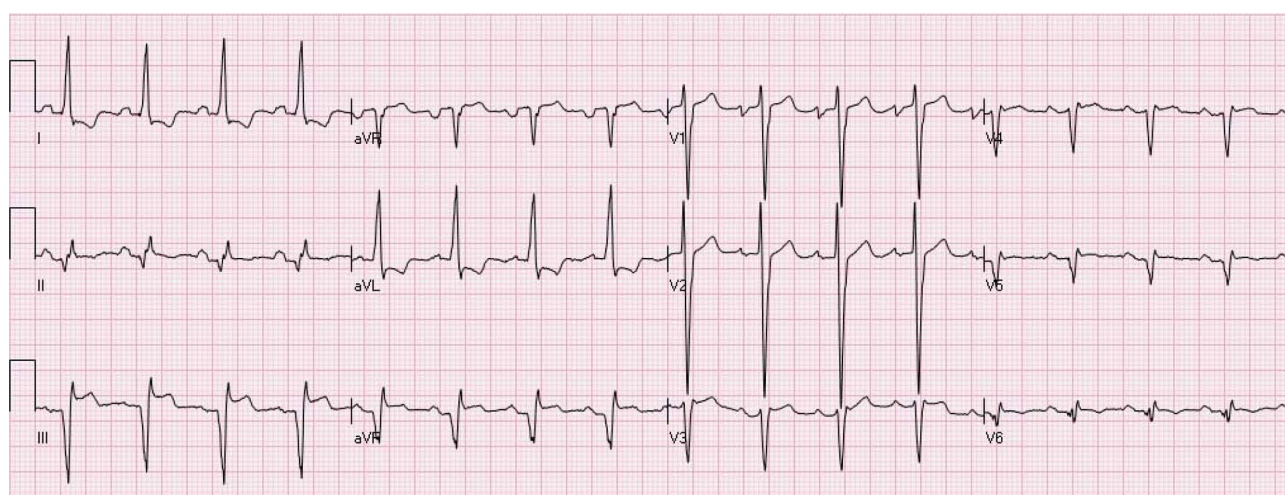


Figure 12 A patient with ST elevation secondary to aneurysm. There are Q waves in the inferior leads + V5-V6 and tall R waves in V1-V2, secondary to old inferolateral ST elevation myocardial infarctions. There is elevation of the ST segments in the inferior leads associated with reciprocal ST depression in leads I and aVL. The electrocardiogram (ECG) is compatible with both acute evolving inferior ST elevation myocardial infarctions or aneurysm. Comparison to previous ECG showed that this pattern is chronic and compatible with aneurysm.

and frequently is mistaken for true STEMI. Some forms of NISTE could show wide variations in the magnitude and extent of STE (for example, the Brugada syndrome or early repolarization). These changes over time; however, differ from the typical pattern of ECG evolution after STEMI.

CONCLUSION

The physician receiving the patient with symptoms compatible with STEMI at the first encounter should make reperfusion decisions as soon as possible after interpreting the initial ECG^[1,3]. This goal was set up because the benefits of reperfusion therapy decline rapidly as the duration of ischemia is prolonged. Rapid interpretation

of the ECG is crucial to shorten door-to-balloon time^[43]. In an attempt to shorten the time to reperfusion, new approaches are currently being tested^[44].

One of the more successful strategies is pre-hospital activation of the coronary catheterization laboratory by emergency medical services. Systems have been established in which in addition to interpreting the transmitted ECG, the interpreting physician can directly communicate with the emergency medical system (EMS) team or even the patient *via* a mobile phone^[44]. The pre-hospital activation strategy is associated with a door to balloon time reduction of 15.4 min^[44]. In the systems used in the United States, on the other hand, the electrocardiographer does not have the advantage of a face-to-face history taking and physical examination of the patient. In addition, even

if previous ECGs are stored in the (electronic) medical records, as a result of privacy issues, ECGs are transmitted without any identifier details, including names. Hence, the interpreter is not able to compare the transmitted ECG with preceding ECGs, even if they are readily available. Diercks and colleagues have shown an improvement in mortality for patients in whom pre-hospital activation system was used (6.7%), *vs* in patients without prehospital activation (9.5%)^[45]. Although such approach might increase the sensitivity of detecting STE, the specificity and false activation remains a problem. The reported false-positive rate ranges from 5.6% to 25%^[43,46-48]. These data suggest there is significant room for improvement.

To evaluate the capability of experienced experts in ECG reading to differentiate between STEMI to NISTE, 15 experienced ECG readers analyzed 116 ECGs showing elevation of the ST segments. The readers were asked whether the catheterization laboratory should be activated for possible STEMI if patients had symptoms suggestive of ACS^[49]. In this set of ECGs, only 7% had adjudicated STEMI and 8 more patients had elevation of the heart muscle markers without clinical indication of STEMI. The number of cases for which acute reperfusion therapy was suggested by each of the ECG experts ranged between 7.8% to 33%. There were wide differences in sensitivity [50% to 100%, (average 75%)] and specificity [73% to 97%, (average 85%)] of the individual readers^[49]. This study suggested that there is a need for refining the criteria for differentiating between NISTE and STEMI in different population setting and that the available criteria for diagnosing STEMI should be refined and standardized in order to maximize the accuracy of ECG interpretation.

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Non-interventional management of resistant hypertension

Michael Doulmas, Costas Tsioufis, Charles Faselis, Antonios Lazaridis, Haris Grassos, Vasilios Papademetriou

Michael Doulmas, Antonios Lazaridis, 2nd Propedeutic Department of Internal Medicine, Aristotle University, 54643 Thessaloniki, Greece

Costas Tsioufis, 1st Cardiology Department, Kapodestrian University, 11527 Athens, Greece

Charles Faselis, Department of Internal Medicine, Veterans Affairs Medical Center and George Washington University, Washington, DC 20422, United States

Haris Grassos, Department of Cardiology, KAT Hospital, 14561 Athens, Greece

Vasilios Papademetriou, Department of Cardiology, Veterans Affairs Medical Center and Georgetown University, Washington, DC 20422, United States

Author contributions: Doulmas M, Tsioufis C and Papademetriou V conceived the manuscript; all the authors drafted parts of the manuscript and revised the whole manuscript.

Correspondence to: Michael Doulmas, MD, PhD, 2nd Propedeutic Department of Internal Medicine, Aristotle University, 49 Konstantinoupolis Street, 54643 Thessaloniki, Greece. michalisdoulmas@yahoo.co.uk

Telephone: +30-2310-992836 Fax: +30-2310-992834

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be clarified. In an effort to manage patients with resistant hypertension appropriately, clinical doctors are still racking their brains in order to find the best therapeutic algorithm and surmount the substantial difficulties in controlling this clinical entity. This review aims to shed light on the effective management of resistant hypertension and provide practical recommendations for clinicians dealing with such patients.

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Key words: Resistant hypertension; Antihypertensive drugs; Adherence; White coat hypertension; Secondary hypertension

Core tip: Patients with resistant hypertension are exposed to high cardiovascular risk and proper medical management continues to puzzle clinicians. The appropriate management of resistant hypertension is still elusive. This review provides practical recommendations for the management of resistant hypertension, aiming to help primary care physicians. It also highlights that the therapeutic scheme should always match the patient's profile in terms of safety, tolerability and effectiveness.

Abstract

Hypertension is one of the most popular fields of research in modern medicine due to its high prevalence and its major impact on cardiovascular risk and consequently on global health. Indeed, about one third of individuals worldwide has hypertension and is under increased long-term risk of myocardial infarction, stroke or cardiovascular death. On the other hand, resistant hypertension, the "uncontrollable" part of arterial hypertension despite appropriate therapy, comprises a much greater menace since long-standing, high levels of blood pressure along with concomitant debilitating entities such as chronic kidney disease and diabetes mellitus create a prominent high cardiovascular risk milieu. However, despite the alarming consequences, resistant hypertension and its effective management still have not received proper scientific attention. Aspects like the exact prevalence and prognosis are yet to

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INTRODUCTION

Despite impressive advances in the area of therapeutics, cardiovascular disease (CVD) continues to be the leading cause of death, even in the 21st century^[1,2]. Among causative factors, hypertension carries the greatest risk for cardiovascular (CV) mortality and morbidity. With a prevalence of around 30% worldwide, individuals with high blood pressure have a five times greater risk of suffering

a debilitating stroke, whereas 50% of hypertensives will suffer from ischemic heart disease and around 7.0 million people will die each year^[1,2]. Surprisingly, a considerable number of individuals with arterial hypertension remain undertreated or uncontrolled despite a combination of at least three antihypertensive drugs (including a diuretic), thus meeting the classical criteria of resistant hypertension (RH). Furthermore, since hypertension begets hypertension and hypertension worsens vascular disease and vice versa, it is reasonable to consider RH a vascular emergency. In fact, prior to the advent of pharmacological therapy, these are the patients that would progress to an accelerated and malignant hypertension phase with dire consequences.

DEFINITION-PREVALENCE

According to the seventh report of the Joint National Committee 7 (JNC7), RH is defined as the lack of control of blood pressure (BP) or BP above the therapeutic goal despite the use of three antihypertensive drugs, including a diuretic, at optimal doses^[3]. BP controlled with more than three antihypertensive medications is also included in the most recent definition of the American Heart Association (AHA)^[4]. A more recent definition comes from the latest guidelines for the management of arterial hypertension of the European Society of Hypertension (ESH) and European Society of Cardiology (ESC). According to the authors, RH is defined as arterial hypertension above the therapeutic goal of systolic blood pressure (SBP) (140 mmHg) and diastolic blood pressure (DBP) (90 mmHg), and resistant to treatment despite the implementation of appropriate lifestyle measures and a combination therapy of three antihypertensive drugs, including a diuretic, at adequate doses^[5]. With recent publicity surrounding RH, more and more studies indicate increasing frequency, but true prevalence remains largely unknown. Data from relatively small studies published so far indicate ranging prevalence from 5% in the general population to 50% in nephrology clinics^[6-8]. However, more recent data from the United States and Spain suggest a prevalence of resistant hypertension of approximately 10%^[9-10]. Yet, even these data are questionable due to methodological limitations^[11].

PROGNOSIS

Similarly to prevalence, the prognosis of patients with RH remains an area widely understudied. It is well established that arterial hypertension and CV risk are a very tight dual complex and that CV morbidity and mortality is directly related to BP levels^[12]. It thus seems rational to assume that patients with RH presenting with long-standing, uncontrolled, high BP might be at a much higher CV risk^[13]. This assumption is further supported by the fact that most patients with RH have many other CV risk factors, such as chronic kidney disease (CKD), obstructive sleep apnea (OSA), diabetes or left ventricu-

lar hypertrophy (LVH)^[4,8]. Although rationally sound, only small clinical studies and observational cohorts have tried to give a more concrete element to this relationship, demonstrating up to a six fold higher CV risk for patients with RH^[14-20]. Therefore, ESH and ESC guidelines incorporated RH as a condition associated with a high risk of CV and renal events^[5].

The most significant information in this field comes from two recent studies. In a large retrospective observational study of more than 200000 patients and a median follow-up of 3.8 years, it was found that CV event rates were almost 50% higher in patients with RH compared to those without RH^[21]. Although very important, this large study suffers from the inherent drawbacks of retrospective analysis of data stored in large databases. A more accurate estimation of RH-associated CV morbidity comes from a meticulous study of almost 2000 hypertensive patients with a mean follow-up of 3.9 years^[22]. It was found that RH was associated with a 2.2-fold increased risk of CV morbidity compared to control patients without RH. However, the accurate risk while being uncontrolled and the exact benefit from efficiently controlling RH are yet to be found.

MANAGEMENT

Given the relatively high prevalence of RH and the presumably high CV risk of this condition, proper management of the affected individuals should be promptly established. In general, the ideal approach of a patient with RH should focus on two goals, with the primary being identification, careful evaluation and, if possible, reversal of contributing factors, followed by an effective individualized drug regimen.

After ensuring that treatment resistance is not due to improper office BP measurement, especially in elderly patients, the astute physician has to exclude other causes of “pseudo-resistance”. The possibility of secondary hypertension should be examined, probably evaluating target-organ damage (TOD). Practical recommendations for a step-by-step approach are presented in detail.

WHITE COAT HYPERTENSION

White coat hypertension is a commonly encountered factor that must always be ruled out. Several small studies pointed towards an increased prevalence of white coat hypertension among patients with RH^[15,16,23] and a large study of more than 8000 patients with apparent RH unveiled the magnitude of the white coat effect^[10]. Using ambulatory BP monitoring, it was found that only 62.5% of patients with office RH actually had true RH, while the remaining 37.5% had white coat hypertension^[10]. Apart from ambulatory BP monitoring, white coat hypertension may be excluded with the use of home BP measurements as well. In a large 20 year study of more than 2300 patients with office RH, white coat hypertension was identified in approximately 30% of study participants, mainly through home BP monitoring^[24].

Adherence to therapy

Poor adherence to prescribed medication is a major problem in the cardiovascular field. A population study of about half a million patients in Italy revealed that 33% discontinued antihypertensive drugs within 6 mo of treatment initiation and the discontinuation rate reached 50% at 5 years post-treatment^[25], with obvious detrimental consequences. Indeed, continuation of antihypertensive drugs is associated with a 37% reduction of CV events in hypertensive patients^[26]. Moreover, the CV risk is 25% lower in patients with high compliance compared to those with low compliance with antihypertensive agents^[26]. The problem of persistence with antihypertensive therapy in patients with RH was recently highlighted in a small study from Germany. Among 108 patients with true resistant hypertension, it was found that more than half of them were non-adherent to therapy; more impressively, among non-adherent patients, 30% were completely non-adherent and 56% were taking less than half of the prescribed drugs^[27]. Although the study was small, it was well-designed and used state-of-the-art toxicological methods to assess antihypertensive drug levels, indicating that poor treatment adherence is actually exaggerated in patients with apparent RH and is a major problem. Another just published study of 339 patients with RH assessing serum levels of antihypertensive drugs confirmed the findings of the previous study since 47% of patients were non-compliant to therapy (either completely or partially)^[28].

Clinical inertia

Clinical or physician inertia in the hypertension field can be defined as the failure of treating physicians to initiate, intensify or change therapy when BP values are above the therapeutic goal. It has long been recognized that physicians are often reluctant to appropriately manage high blood pressure levels and do not start, intensify or switch antihypertensive therapy in about one third of occasions^[29,30], reaching 50% in patients with comorbidities^[31,32]. Clinical inertia seems to play a major role in RH. In a recent study of more than 3500 patients with diagnosed RH, treatment intensification (dose increase or drug addition) occurred in only 21.6% of visits with elevated BP^[33].

The observation that treatment intensification occurs in only one of five clinical visits is shocking and deserves to be examined in-depth, to be highlighted and appropriately addressed. First, it seems to reflect everyday clinical practice since the vast majority (99.5%) of clinical visits in the latter study was performed in primary care (family practice, internal medicine and obstetrics/gynecology). However, the big surprise comes from another finding of this study and regards diuretic use: instead of intensifying diuretic use by dose increment, the study reported that diuretic use was actually reduced by 15% at one year after the diagnosis of RH. Another finding of this study confirms the importance of increasing treatment intensity: treatment intensification was associated with a 64% increase in BP control at 1 year post-RH diagnosis.

Drugs inducing hypertension

A long list of drugs (either prescribed or over-the-counter) and exogenous agents result in BP elevation and consequently either induce hypertension or contribute to resistance in drug therapy. Drug-induced hypertension is common and among the main causes of treatment resistance^[34]. The most frequent agents associated with drug-induced BP elevation are without any doubt non-steroidal anti-inflammatory drugs which are widely prescribed for a variety of conditions and are also available over-the-counter^[35-37]. Other common causes include oral contraceptives, hormone replacement therapy, and sympathomimetics^[38-40]. Special attention needs to be drawn to drugs that are not commonly used but are essential for the treatment of specific conditions: erythropoietin for the treatment of CKD-associated anemia and myelodysplastic syndromes, cyclosporine and tacrolimus for organ transplantation, mineralocorticoids for adrenal insufficiency, glucocorticoids for a wide variety of conditions, and some newer anti-neoplastic drugs (VEGF-inhibitors and tyrosine-kinase inhibitors)^[41-43]. Finally, illicit drugs and herbal supplements must not be forgotten as causes of treatment resistance in hypertensive patients^[44,45].

Some points regarding drug-induced BP elevation need to be highlighted. First is the heterogeneity of BP response to the above mentioned agents. Some patients experience excessive BP elevation while other patients exhibit little if any BP elevation. Then, the necessity of the administered drugs inducing BP elevation dictates management: (1) when the drug is not essential it can be withdrawn; (2) when the drug is essential and replacement with another less susceptible drug or dose reduction seems possible it can be tried; and (3) when the drug is essential and cannot be replaced or down-titrated then the best solution seems to be to treat the elevated BP with the more appropriate antihypertensive drugs for each condition. Last, but most important, is the identification of drug-induced BP elevation. Despite its high frequency and the easiness of its recognition, treating physicians often miss the opportunity of recognizing iatrogenic hypertension, a common identifiable cause of treatment resistance.

Secondary hypertension

Secondary forms of hypertension are not rare and are frequently associated with treatment resistance unless the etiological factor is removed. The list for secondary hypertension is long and includes a wide variety of conditions^[46]; however, a detailed presentation of these causes is outside the scope of the current review. Special attention should be given to the most common causes of secondary hypertension: primary hyperaldosteronism, renal parenchymal disease, renovascular disease and obstructive sleep apnea^[34]. For example, in a large study of more than 2000 patients with RH, primary hyperaldosteronism was identified in approximately 11% of study participants^[24].

The astute physician, however, needs to know all the forms of secondary hypertension, recognize their pre-

senting symptoms, be familiar with the tests required to establish or rule out their diagnosis, and effectively treat these conditions. It has to be noted that a lot of experience is required to raise suspicion and unveil secondary forms of hypertension because there is a two-edged sword: either miss the diagnosis of a secondary form of resistant hypertension or perform several unnecessary tests without an obvious reason and with a tremendous cost. It therefore seems rational to recommend referral to a specialized center when the suspicion of a secondary form is raised by primary care physicians^[5].

Target organ damage

The recent 2013 guidelines for the management of arterial hypertension recommend the recognition of TOD in patients with arterial hypertension^[5]. The reason for this recommendation lies mainly in a more complete and accurate estimation of CV risk and the subsequent reclassification of patients with low or intermediate risk to a higher risk level, as well as the specific treatment of the various forms of TOD with appropriate antihypertensive drugs. TOD is common in patients with RH and more frequently recognized compared to patients without RH. Indeed, left ventricular hypertrophy, arterial stiffness, microalbuminuria, diastolic dysfunction and chronic kidney disease are more common in patients with RH than in control patients^[47-51]. The association between RH and TOD represents a “chicken-egg” question: is it the RH that results in TOD or is hypertension more difficult to control in patients with TOD? Although available data does not allow for definite conclusions, it seems that this association is bi-directional and both types of association occur in patients with RH.

Although we do not wish to dispute the importance of identifying TOD, we believe that it is of marginal clinical significance in patients with RH. Our belief is mainly for two reasons: (1) patients with RH are already at very high CV risk, due not only to hypertension but to the frequent existence of comorbidities as well; and (2) more importantly, patients with RH are already being aggressively treated with the majority of available means of the antihypertensive therapeutic armamentarium and the recognition of TOD is not likely to alter the therapeutic regime. Therefore, the quest for TOD in patients with RH seems to be currently of little if any clinical significance.

NON-PHARMACOLOGICAL APPROACH

Lifestyle factors (obesity, excessive salt intake, physical inactivity, smoking, increased alcohol consumption) contribute significantly to the multifactorial etiology of treatment resistance and are prominent therapeutic targets during assessment of patients with RH^[4,8]. Thus, common lifestyle modifications such as dietary weight loss, salt restriction, increased physical activity, smoking cessation and moderation of alcohol intake are recommended and should be always incorporated in the therapeutic plan of individuals with RH^[4,8]. However, the evidence behind

these recommendations is not always strong and often relies on potential benefits and the lack of harm.

Several lines of evidence from epidemiological longitudinal studies and randomized clinical trials indicate that hypertension is more difficult to control in obese patients and requires more antihypertensive drugs^[52-55]. In a recent report from NHANES, obesity was identified as a strong and independent predictor of apparent treatment-resistant hypertension^[56]. Obesity-induced treatment resistance might be mediated by sympathetic activation, volume expansion, aldosterone excess and obstructive sleep apnea^[57-59]. Although the benefits of weight loss on BP are not questioned, the impact of weight reduction (through lifestyle modification, pharmacological agents or bariatric surgery) on BP in patients with RH is poorly studied and needs to be confirmed by properly designed studies. Likewise, smoking cessation and alcohol moderation have not been adequately studied in RH, despite the undisputable benefits of these changes in lifestyle factors.

The paramount importance of salt restriction in patients with RH was recently highlighted. A small, randomized, cross-over study of 12 patients with RH evaluated the effects of a low and high sodium diet on BP^[60]. It was found that a low sodium diet was associated with a substantial reduction of office systolic and diastolic BP by 22.7 and 9.1 mmHg, respectively. Of major importance, a similar reduction in ambulatory BP was observed as well (20.1/9.8 mmHg), both during the day and night, despite the fact that ambulatory BP reduction tends to be significantly lower than office BP reduction^[61].

Fitness and increased exercise capacity are associated with significant morbidity and mortality benefits in patients with hypertension, prehypertension and high normal blood pressure, even in elderly patients^[62-66]. The significance of regular exercise in patients with RH was recently demonstrated. A randomized study of 50 patients with RH assessed the effects of a treadmill exercise program for 8 to 12 wk^[67]. It was found that regular aerobic exercise is associated with a significant reduction in ambulatory BP by 6/3 mmHg. Another small study of 16 patients with RH points towards significant benefits of heated water-based exercise^[68] but further studies are needed to confirm these preliminary findings.

DRUG TREATMENT

After all contributing factors have been carefully assessed and effectively managed, treatment of true RH, whether pharmacological or not, relies on inhibiting the pathophysiological pathways resulting in BP elevation. Activation of the renin-angiotensin system (RAS), sympathetic nervous system (SNS) overactivity and intravascular volume expansion are the three cardinal pharmacological targets in the therapeutic algorithm of an individual with RH^[4,8,13,34]. The means to achieve these targets cover a broad spectrum of agents, including diuretics, angiotensin converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARBs), calcium channel blockers (CCBs), beta blockers (BBs), alpha blockers, centrally acting drugs

and other potent vasodilating agents. The most prescribed drug categories among patients with RH are RAS inhibitors (ACEi and ARBs), diuretics, CCBs and BBs. A recent report of more than 140000 patients with RH included in the large Medstat database revealed that 96.2% of patients were on RAS inhibitors, 93.2% on diuretics, 83.6% on CCBs, and 80% on BBs^[69]. Chronotherapeutics might also play a role and bedtime administration of one drug or one dose might be beneficial in terms of both BP control and outcomes^[70,71]; however, more solid evidence is needed before the wide generalization of this approach.

We propose a therapeutic approach of a step-by-step addition of antihypertensive drug classes in patients with RH. This approach is based on the pathophysiology of RH, the properties of antihypertensive drugs, the safety profile and the efficacy of each class of agents in RH. It has to be noted, however, that available data in RH is scarce and limited with the vast majority of antihypertensive drugs. Therefore, the proposed approach is more scientifically sound than evidence-based. Prospective studies are needed to evaluate the efficacy, safety and utility of this approach.

Triple therapy

In general terms, combining available agents is the cornerstone of treatment of RH. The challenge, however, rests upon constructing a regime that will be both effective, in terms of blocking the majority of the implicated pathophysiological pathways, and individualized, according to the patient's profile, lifestyle, comorbidities or even financial limitations. Moreover, the optimal combination should be well tolerated by the patient, with minimal adverse events to ensure long-term adherence to therapy.

Taking into account the above considerations, a triple combination of an ACEi or ARB along with a diuretic and a CCB seems to be a reasonable regime when approaching a patient with RH for the first time. This combination is scientifically sound, widely used in everyday clinical practice, and should be applied in high doses as the first therapeutic step in patients with true RH after all forms of "pseudo-resistance" have been excluded. This combination might be applied in terms of switching previous therapy or of treatment intensification in patients already using this combination in lower doses. The proposed triple combination has several advantages in terms of efficacy, safety profile, adherence to therapy and financial costs.

In terms of efficacy, this combination seems very attractive. Inhibition of the RAS system with an ACEi or an ARB is a very useful tool to subdue high BP, especially in patients with concomitant CKD, heart failure, myocardial infarction, diabetes mellitus and most forms of TOD^[5]. Combining a RAS inhibitor with a CCB or a diuretic is a very popular and scientifically sound choice, especially for black people and the elderly in whom CCBs and diuretics are of particular value^[3]. Several studies have demonstrated the CV benefit of prescribing these

two combinations and, as a matter of fact, many fixed-dose combinations have been on the market for several years^[72].

In terms of safety, RAS inhibitors are known to attenuate the most common adverse events of the other two classes: peripheral edema induced by calcium antagonists and hypokalemia induced by diuretics^[5,72]. In terms of persistence in therapy and cost, dual fixed combinations (RAS inhibitors plus thiazides or calcium antagonists) have been available for many years on the market and physicians are already familiar with their use. Using fixed combinations increases adherence to antihypertensive therapy^[73]. Moreover, fixed combinations are usually cheaper than the administration of each drug separately and since drugs comprising these combinations are already off-patent, fixed combinations are preferred from the financial point of view. Triple fixed combinations were recently introduced in the market and are likely to improve patients' adherence to therapy with obvious health and financial benefits^[74].

In clinical practice, it is not unusual to see patients referred for RH who are actually receiving inappropriate combinations or low doses of appropriate combinations. This clinical observation provides the basis for the first step of the proposed therapeutic approach. The combination of ACEi with ARBs provides a very good example of inappropriate or non-preferred combinations. This combination was very popular during the last decade despite its moderate efficacy on BP reduction compared to other combinations, mainly due to expectations for potential benefits on target organ protection, especially cardioprotection and nephroprotection. The dual inhibition of the renin-angiotensin system suffered a lethal kick by the ONTARGET study, in which the combination did not confer any additional benefits compared to RAS monotherapy and was associated with more adverse events^[75]. More recently, two other studies seem to have put the final nail in the coffin. The NEPHRON-D study found no benefit in patients with CKD^[76] and the ALTI-TUDE study reported similar results for the combination with direct renin inhibitors^[77]. Therefore, guidelines for the management of arterial hypertension strongly recommend avoiding dual RAS inhibition. Everyday clinical practice, however, is cruel. Among 140000 patients with RH included in a large database, 15.6% were treated with ACEi plus ARBs^[69].

Overall, we believe that a triple combination of RAS-inhibitors, CCBs and diuretics in high doses should be tried in all patients with true resistant hypertension before other drugs are added. Certainly, exceptions apply for this combination as well, such as patients that are intolerant of one or more drugs included in this combination, especially in high doses, since it is known that high doses of CCBs and diuretics are associated with an increased prevalence of peripheral edema and hypokalemia, respectively. Furthermore, CCBs are relatively contraindicated in patients with chronic heart failure and it is better to substitute with beta blockers. Similarly, BB should be pre-

ferred in patients with RH and symptomatic CAD.

Thiazide diuretics

Among the antihypertensive agents available in our quiver, emphasis should be given to diuretics. This is due to the fact that volume expansion seems to be the most implicating pathophysiological cause of RH. In fact, several lines of evidence have demonstrated that over 60% of patients could gain better BP control with proper diuretic therapy. Thus, adding a diuretic, increasing the dosage of the existing one or even changing the prescribed diuretic should be the mainstay of any treatment modification^[4,8,13,34].

More specifically, hydrochlorothiazide should be used at adequate doses of up to 50 mg/d, assuming a satisfactory renal function with an estimated glomerular filtration rate (eGFR) > 40-50 mL/min per 1.73 m². Chlorthalidone has proved to be similarly or more effective; however, it is not widely prescribed due to its limited availability in fixed dose combinations. Whenever renal insufficiency is present, as defined by levels of eGFR < 40 mL/min per 1.73 m², loop diuretics should take their place in the therapeutic regime. Due to their relatively short duration of action, furosemide or bumetanide should be given twice or even thrice daily, whereas torsemide with its longer half-life can be given only once per day.

During the last five years, a vivid discussion has taken place regarding the comparison of hydrochlorothiazide with chlorthalidone^[78,79]. Chlorthalidone is long-acting, almost twice as potent as hydrochlorothiazide at the same dose, and has a better 24 h antihypertensive profile^[80,81]. In addition, chlorthalidone was used in the ALLHAT study and proved to be equal to other antihypertensive drugs^[82], while hydrochlorothiazide and bendroflumazide were used in the ACCOMPLISH and the ASCOT trials respectively and proved to be inferior to comparison therapy^[83,84]. Moreover, an indirect comparison of chlorthalidone with hydrochlorothiazide in the MRFIT study pointed towards the superiority of chlorthalidone^[85]. However, further studies are needed in this field and specifically in patients with RH before definite conclusions can be drawn.

Mineralocorticoid inhibitors

Activation of the RAS and consequently aldosterone production is a very common phenomenon in RH and a principal therapeutic target^[86,87]. Aldosterone excess can be efficiently blocked by mineralocorticoid receptor antagonists (spironolactone and eplerenone). Several small clinical studies during the last decade proved the efficacy of spironolactone in reducing BP in patients with RH by approximately 20/10 mmHg for systolic and diastolic BP respectively^[88-93]. This unprecedented BP reduction in patients with RH seems to be independent of baseline aldosterone levels and more pronounced in specific populations such as obese people and those with obstructive sleep apnea. The beneficial effects of spironolactone were confirmed in the ASCOT study, in which spironolactone was used as fourth line therapy in 1411

patients of both treatment arms (diuretic + BBs vs ACEi + CCBs) following the addition of doxazosin (an alpha-blocker). Indeed, BP was reduced by 21.9/9.5 mmHg with spironolactone in this study^[94]. Of note, patients in the first arm of the study were by definition RH as BP remained uncontrolled despite the use of 3 antihypertensive drugs, including a diuretic.

The enthusiasm for spironolactone use was somehow dampened by the findings of two recent studies. The ASPIRANT study, a double-blind, randomized, placebo-controlled study evaluated the effects of spironolactone in 117 patients with RH^[95]. It was found that daytime ambulatory BP reduction with spironolactone was only 5.4/1.0 mmHg. In another, randomized, double-blind, placebo-controlled study of 119 diabetic patients with RH, the average ambulatory daytime BP reduction was 8.9/3.7 mmHg^[96].

Another significant concern regards the risk of hyperkalemia and renal function deterioration. Patients with RH are already on RAS inhibition and CKD is frequently encountered in such patients, thus increasing the risk of hyperkalemia. Therefore, extreme caution is required, especially at treatment initiation, on renal function and potassium levels. Although a specific algorithm for RH has not been yet proposed, the recommendations of AHA regarding spironolactone use in patients with heart failure seem prudent and might apply for patients with RH as well^[97]. In case of gynecomastia with spironolactone, usually seen at doses above 25 mg/d, eplerenone, a more selective agent, is well tolerated and effective^[98]. It has to be noted, however, that larger doses of eplerenone are usually required for the same antihypertensive effect and the significantly higher cost of eplerenone limits its use in RH.

Other antihypertensive drugs

Treatment guidelines recommend maximizing diuretic therapy, either by using chlorthalidone or by adding mineralocorticoid antagonists or both as needed. Are these recommendations implemented in primary care? The truth in everyday clinical practice is once again cruel. Among more than 5 million hypertensive patients included in the Medstat database, 140000 were using four or more antihypertensive drugs, fulfilling the criteria of RH. The rates of chlorthalidone and mineralocorticoid antagonist use were disappointingly low: 3% for chlorthalidone and 5.9% for aldosterone antagonists^[69].

However, even in cases where chlorthalidone or spironolactone are used, a considerable proportion of individuals with RH still have uncontrolled BP. These patients will need a fifth medication with the rationale of implementing an agent with a different mechanism of action compared to the already used regime. Blockade of SNS hyperactivity could be a solution to this therapeutic dilemma. BBs are particularly effective when concomitant coronary artery disease or congestive heart failure exists. Another reasonable approach would be to combine a BB along with an alpha blocker such as doxazosin, as data has shown that it is possible to achieve a more potent an-

tihypertensive effect.

Even then, a handful of patients will still resist antihypertensive treatment, thus rendering the evaluation of the role of centrally acting antihypertensive agents (clonidine, moxonidine, methyldopa) or potent vasodilators (hydralazine, minoxidil) as the next step. Although significantly effective in lowering BP, the increased incidence of side effects, their poor tolerability and the lack of concrete data make the implementation of these agents always with caution^[3]. Finally, limited data support the antihypertensive action of a non-dihydropyridine CCB complementary to a dihydropyridine one^[99]; however, data is limited and requires confirmation in patients with RH.

Failure of drug therapy

After all pathophysiological pathways have been blocked and most appropriate pharmaceutical efforts and combinations have been made, it is evident that a reasonable number of patients will still retain remarkably high levels of BP. Rendering our whole medical armamentarium ineffective or poorly tolerated, this group of patients are undoubtedly the permanent headache of clinicians working in primary care. Advanced help should be sought and these patients should be referred to a hypertension specialist as new and more efficacious treatments, mostly in the interventional sector, come into sight^[100-110].

CONCLUSION

Whereas arterial hypertension comprises one of the most extensively studied entities in the medical literature and while a huge collection of antihypertensive agents is available in our therapeutic armamentarium, surprisingly, a considerable number of patients do not achieve optimal BP control. In fact, individuals with RH continue to be exposed to high CV risk and proper medical management continues to puzzle clinicians. In any case, a proper initial approach should include detailed evaluation, exclusion and correction of other contributing factors, along with confirmation of true resistant hypertension. Consequently, an appropriate drug regime should be sought, based on blocking the mechanisms involved in the pathophysiology of RH. At the same time, the therapeutic scheme should always match the patient's profile in terms of safety, tolerability and effectiveness. Until new drug regimens are available, newer techniques of interventional management will keep the promise to radically transform our therapeutic approach towards RH.

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Mitochondria-targeted agents: Future perspectives of mitochondrial pharmaceuticals in cardiovascular diseases

Thekkuttuparambil Ananthanarayanan Ajith, Thankamani Gopinathan Jayakumar

Thekkuttuparambil Ananthanarayanan Ajith, Department of Biochemistry, Amala Institute of Medical Sciences, Thrissur 680 555, Kerala, India

Thankamani Gopinathan Jayakumar, Department of Interventional Cardiology, Amala Cardiac Centre, Thrissur 680 555, Kerala, India

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Correspondence to: Thekkuttuparambil Ananthanarayanan Ajith, PhD, Professor, Department of Biochemistry, Amala Institute of Medical Sciences, Amala Nagar, Thrissur 680 555, Kerala, India. taajith@rediffmail.com

Telephone: +91-487-2304116 Fax: +91-487-2307969

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Abstract

Mitochondria are one of the major sites for the generation of reactive oxygen species (ROS) as an undesirable side product of oxidative energy metabolism. Damaged mitochondria can augment the generation of ROS. Dysfunction of mitochondria increase the risk for a large number of human diseases, including cardiovascular diseases (CVDs). Heart failure (HF) following ischemic heart disease, infantile cardiomyopathy and cardiac hypertrophy associated with left ventricular dilations are some of the CVDs in which the role of mitochondrial oxidative stress has been reported. Advances in mitochondrial research during the last decade focused on the preservation of its function in the myocardium, which is vital for the cellular energy production. Experimental and clinical trials have been conducted using mitochondria-targeted molecules like: MnSOD mimetics, such as EUK-8, EUK-134 and MitoSOD; choline esters of glutathione and *N*-acetyl-L-cysteine; triphenylphosphonium ligated vitamin E, lipoic acid, plastoquinone and

mitoCoQ₁₀; and Szeto-Schiller (SS)- peptides (SS-02 and SS-31). Although many results are inconclusive, some of the findings, especially on CoQ₁₀, are worthwhile. This review summarizes the role of mitochondria-targeted delivery of agents and their consequences in the control of HF.

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Key words: Cardiovascular diseases; Oxidative stress; Antioxidant; Electron transport chain; Mitochondrial medicine; Heart failure

Core tip: Dysfunction of mitochondria increases the risk for a large number of human diseases, including cardiovascular diseases. Heart failure (HF) following ischemic heart disease, infantile cardiomyopathy and cardiac hypertrophy associated with left ventricular dilations are some of the cardiovascular diseases in which the role of mitochondrial oxidative stress has been reported. Recent reports on chronic HF followed by ischemic heart disease suggested a reduced supply of energy necessary for the contractile function of cardiomyocytes. Since mitochondrial damages are central to the pathophysiology of HF, various approaches are used to target compounds at mitochondria alone or adjunct to standard therapies.

Ajith TA, Jayakumar TG. Mitochondria-targeted agents: Future perspectives of mitochondrial pharmaceuticals in cardiovascular diseases. *World J Cardiol* 2014; 6(10): 1091-1099 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i10/1091.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i10.1091>

INTRODUCTION

Although substantial improvements were made in the treatment of cardiovascular events during the last decade, cardiovascular disease (CVD), such as atherosclerosis,

ischemic heart disease (IHD), heart failure (HF), stroke and hypertension, still remain one of the major challenges to humans. HF is a leading cause of morbidity and mortality in industrialized countries. It is also a growing public health problem, mainly because of the aging of population and an increase in prevalence in the elderly. In developing countries, around 2% of adults suffer from HF; the prevalence is found to be increased to approximately 6%-10% over the age of 65^[1]. The mechanisms of HF are complex and multifactorial. Common causes of HF include myocardial infarction (MI) and other forms of IHD, valvular heart disease and different types of cardiomyopathies. A study of healthy adults in the United States reported that IHD increases the risk factors of HF by approximately 62%^[2]. No curative treatment is currently available for HF. The existing therapies for HF are able to relieve symptoms but are unable to reverse molecular changes that occur in the cardiomyocytes. A reduced supply of energy necessary for the contractile function of cardiomyocytes can explain the chronic HF followed by IHD^[3]. This may probably be due to the increased production of oxygen radicals with or without preserving the antioxidant status in the cardiomyocytes^[4].

The primary factor that initiates the dysfunction of mitochondria has been proposed to be the defects in oxidative phosphorylation (OXPHOS) which can further enhance the production of reactive oxygen species (ROS) and eventually destroy the mtDNA^[5]. Since slowly dividing/postmitotic cardiac myocytes are highly dependent on energy from OXPHOS, the cardiac myocardium will be affected, especially when the proportion of the damaged mitochondria is considerably high, as evidenced in HF^[3]. Hence, challenging mitochondrial dysfunction remains one of the main streams of mitochondrial research that is primarily focussed on alleviating the organ damage associated with CVD. In spite of experimental evidence to support the role of mitochondria-mediated antioxidant therapy to alleviate the ROS-mediated injury in CVD, clinical studies are fragmentary. Many antioxidant molecules are designed and evaluated in clinical and experimental trials to stop the deterioration of mitochondrial function but only a few achieve success. Hence, mitochondrial-targeted antioxidant therapy for CVDs is a controversial field and warrants further research. It is worth knowing the scope for mitochondrially-mediated interventions in the conventional therapeutic regimen in order to render complete protection for early stages of CVD to result in protection for HF. This review discusses the mitochondria-targeted delivery of agents to alleviate the decline of myocardial function in CVD.

FORMATION AND DAMAGE INDUCED BY REACTIVE OXYGEN SPECIES IN THE MITOCHONDRIA OF CARDIOMYOCYTES

Mitochondria play a key role in cardiac energy balance. Energy for cardiomyocytes is solely met from mitochondrial OXPHOS. Moreover, mitochondria are involved

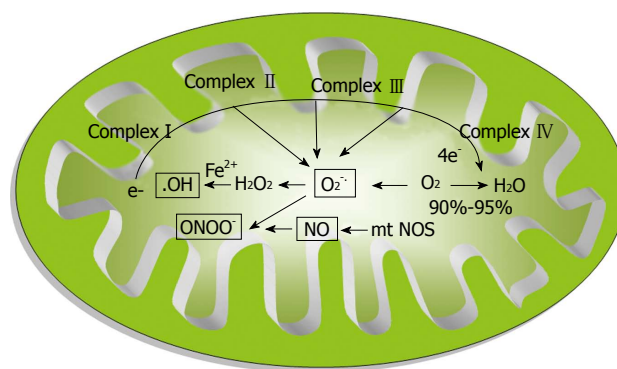


Figure 1 Formation of various reactive oxygen species in mitochondria. HO·: Hydroxyl radical; O₂^{·-}: Super oxide anion radical; ONOO·: Peroxynitrite; mtNOS: Mitochondrial-specific nitric oxide synthase; NO: Nitric oxide.

in maintaining the fine regulatory balance between Ca²⁺ concentration and production of ROS and nitric oxide (NO). The majority of cellular oxygen (O₂) that enters into mitochondria is reduced to water in the mitochondrial respiratory chain, whereas a fraction of all O₂ consumed can be converted to potentially cytotoxic ROS, such as superoxide anion radical (O₂^{·-}), indicating that the mitochondrion itself is the source of ROS^[6]. Any factor that affects the flow of electrons (e⁻) in the electron transport chain (ETC) can result in the leakage of e⁻ to O₂, leading to the formation of O₂^{·-}. The O₂^{·-} is a primary radical that could produce other ROS, such as hydrogen peroxide (H₂O₂) and hydroxyl radicals (·OH), in the failing myocardium. The ·OH is generated by the reduction of H₂O₂ in the presence of endogenous iron and copper by means of the Fenton reaction. Copper and iron are found to be mobilized following myocardial ischemia. Chevion *et al*^[7] reported a 8 to 9-fold higher level of copper and iron in the first coronary flow fraction of reperfusion after 35 min of ischemia compared to the pre-ischemic value in isolated rat heart. This was further supported by the observation of Reddy *et al*^[8] that early treatment with deferoxamine, a potent iron chelator, limits the injury related to myocardial ischemia/reperfusion in dogs, probably due to the lesser availability of iron for the Fenton reaction. The production of various ROS in the mitochondrion is given in Figure 1.

The drugs being used in clinical practice, such as statins (decreases ubiquinone), aspirin and valproic acid (sequesters of CoA), doxorubicin and daunorubicin (releases ROS), and acetaminophen (decreases reduced glutathione), will affect mitochondrial energy production and may play a critical role in the development of cardiomyopathy^[6]. Physiologically, increased demand of the organ can favor the generation of free radicals. Sudheesh *et al*^[9] recently reported that isoproterenol-induced acute MI in rat affected the respiratory chain complexes I-IV, mediated through an increase in the ROS level in the cardiomyocytes. Furthermore, the declined antioxidant status in the mitochondria during aging can also provoke mitochondrial dysfunction in cardiomyocytes^[10]. Hypercholesterolemia can also affect mitochondrial functions by declining the mitochondrial membrane potential mediated through

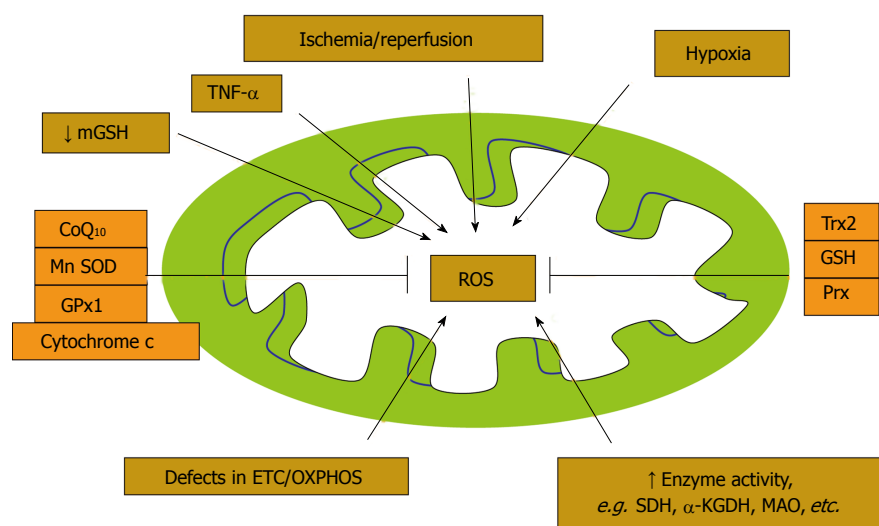


Figure 2 Factors that form and attenuate (antioxidants) reactive oxygen species in mitochondria. SDH: Succinate dehydrogenase; α KGDH: Alpha ketoglutarate dehydrogenase; MAO: Monoamine oxidase; Trx: Thioredoxins; Prx: Peroxiredoxin; OXPHOS: Oxidative phosphorylations; TNF- α : Tumor necrosis factor-alpha; GSH: Reduced glutathione; MnSOD: Manganese containing superoxide dismutase; GPx1: Glutathione peroxidase; CoQ₁₀: Co-enzyme Q₁₀.

the generation of ROS and activation of mitochondrial apoptotic pathway^[11].

Evidence shows that cytokines, such as tumor necrosis factor-alpha (TNF-alpha) and interleukin-6, are important pathological factors in inflammatory responses during the pathological progression of myocardial ischemia/reperfusion and hypertrophy. They are released during chronic inflammation, either in endothelial cells or cardiomyocytes, and inhibit the electron transport through the complex I and complex III-ubiquinone cycle, facilitating the generation of ROS^[12]. Elevated activities of certain mitochondrial enzymes are also directly correlated with the excess production of ROS (Figure 2). The generated ROS is known to induce oxidation of low-density lipoproteins (LDL) in the coronary sinus of patients with dilated cardiomyopathy^[13]. The oxidized LDL is abrogated by binding to the lectin-like oxidized LDL scavenger receptor-1 (LOX-1) on the arterial wall^[14]. Activation of LOX-1 has been related to many pathophysiological events that lead to IHD.

The generated ROS under oxidative stress may contribute to potential mitochondrial damage that induces endothelial dysfunction and promotes leukocyte adhesion, inflammation, thrombosis and smooth muscle cell proliferation^[15]. Among the damage induced by generated ROS at the cellular level, mtDNA remains the major target (Figure 3). mtDNA contains about 16.5 kb of circular double-stranded DNA to encode 13 protein components of the ETC. Mitochondrial function is controlled by the mtDNA, as well as factors that regulate mtDNA transcription and/or replication. A large part of the O₂⁻ that is formed inside the mitochondria cannot pass through the membrane and hence affect the DNA. Since 1988 when the first mutation in mtDNA was established, more than 400 mutations have been identified. The mutations described are either typically 50% to 60% for single, large-scale deletions or 80% to 90% for point mutations

in patients with mitochondrial myopathy and encephalomyopathy^[16]. In general, the majority of pathogenic point mutations are maternally transmitted, whereas large-scale deletions of mtDNA are mostly sporadic. More than 10 different types of deletions have been identified in the mtDNA among these; the 4977-bp deletion is the most prevalent in skeletal muscle, whereas the 7436-bp deletion was detected in the heart of human subjects in their late thirties, with no apparent sex difference^[17]. However, the clinical severity of the disease is usually correlated with the presence of > 80% of the mutated mtDNA in the target tissues^[18]. Furthermore, at the same level, large-scale deletions cause much more severe pathologies than point mutations. The patterns of distribution of the mutated mtDNA and the energy demand of the target tissues are two important factors that determine the pathological outcome of the mutation. HF is frequently associated with qualitative and quantitative defects in mtDNA and is found to increase with the age of human subjects. Recent evidence has suggested that mitochondria have enzymes to proofread mtDNA and fix mutations that may occur due to free radicals^[19].

Often, the damaged mtDNA is degraded by autophagy, whereas mtDNA that escapes the process of autophagy, as observed in atherosclerosis, can induce a potent inflammatory response. Ding *et al.*^[14] demonstrated that the damaged mitochondria induced by ox-LDL can result in the expression of toll-like receptor-9 (TLR-9) on the cell membrane. TLR-9 senses the unmethylated CpG motifs in damaged mtDNA and induces inflammation which is mediated through the pro-inflammatory cytokines. Ding *et al.*^[14] also demonstrated an intense autophagy, TLR-9 expression and inflammatory signals in the aorta of LDL receptor knockout mice when fed with a high cholesterol diet. Use of LOX-1 antibody or the ROS inhibitor apocynin attenuated ox-LDL-mediated autophagy, mtDNA damage and TLR-9 expression.

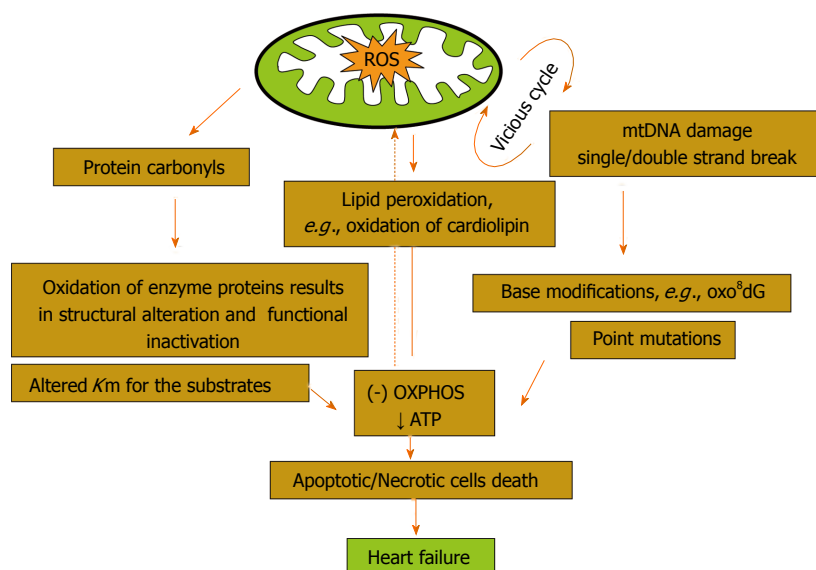


Figure 3 Damage induced by reactive oxygen species in mitochondria. OXPHOS: Oxidative phosphorylations; ROS: Reactive oxygen species.

Experiments using siRNA to DNase II suggested that the DNase II digested mtDNA and protected the tissue from inflammation.

In addition to the mtDNA mutations, damage to protein and lipid molecules in the mitochondrial membrane can contribute to the declined OXPHOS. Cardiolipin, an essential phospholipid present in the inner membrane of mitochondria that serves as a cofactor for a number of critical mitochondrial transport proteins and retains cytochrome c at the inner mitochondrial membrane through the electrostatic interaction, declines during the oxidative damage. Peroxidation of cardiolipin and its release into the cytosol can execute apoptotic cell death^[20]. Amino acids, such as lysine, arginine, glutamic acid, histidine, proline and threonine present in the protein, favor the formation of protein carbonyl or nitration of the tyrosine residues, either by direct oxidation or by the binding of aldehydes that formed from the peroxidation of lipids. Mitochondrial aconitase and adenine nucleotide translocase are highly sensitive to O_2^{2-} ^[21]. ROS-derived lipid hydroperoxide can also initiate the strand breaks and base modifications in mtDNA. Many cardiotoxic stimuli can lead to ROS generation, Ca^{2+} overload of the mitochondrial matrix, and opening of a large, nonspecific channel in the inner mitochondrial membrane, such as permeability transition pore (PTP), finally alter the mitochondrial permeability transition (MPT). Ca^{2+} overload to the mitochondrial matrix can further enhance the generation of ROS. Although the exact mechanism of ROS production is debatable, the effect is probably mediated through Ca^{2+} mediated inhibition on the complex I^[22], III^[23] and IV^[23] of ETC (Figure 4). Ca^{2+} can stimulate the TCA cycle dehydrogenases to increase the production of reduced substrate for OXPHOS^[24] and further increase the rate of respiration as well. Ca^{2+} can also activate mitochondrial nitric oxide synthase to produce NO which in turn inhibits the complex IV^[25]. The simultaneous generation of NO with O_2^{2-} favors the formation of peroxynitrite, one

of the major agents to induce conformational change in many proteins^[26]. MPT dissipates the proton electrochemical potential gradients, depletion of ATP and swelling, as well as rupture of the mitochondria that leads to the release of pro-apoptotic proteins into the cytosol and eventually results in death of cardiomyocytes. Evidence indicates that the activity of complex II is not affected as it is entirely encoded by nuclear DNA, whereas complex IV activity (cytochrome C oxidase), along with complex I, partially encoded by mtDNA genes, are frequently reduced in patients with mtDNA or tRNA mutations^[19]. Mt tRNA gene mutations can also variably affect the activity of respiratory chain complexes.

ANTIOXIDANTS AND PROTECTION OF MITOCHONDRIA IN THE CARDIOMYOCYTES

Mitochondrial oxidative stress resulting from an imbalance between the generation of ROS and the existing mitochondrial antioxidant mechanisms has been described in the pathogenesis of CVDs, including HF^[27]. HF followed by MI can be initiated with the mitochondrial damage and dysfunction that can be ascribed to: (1) increased lipid peroxidation; (2) reduced mitochondrial gene replication, mtDNA copy number and mitochondrial gene transcription; and (3) reduced OXPHOS due to low respiratory chain complex enzyme activities (Figure 5). Therefore, preservation of mitochondrial function is essential. Therapies that are designed to interfere with mitochondrial oxidative stress could be beneficial.

Various molecules are involved in the mitochondrial protection for the myocardium. Among them, tumor necrosis factor receptor-associated protein 1 (TRAP1), a member of the mitochondrial heat-shock family of proteins (70 kDa), has a central role. Overexpressed TRAP1 in the ischemia-like condition preserves ATP levels and

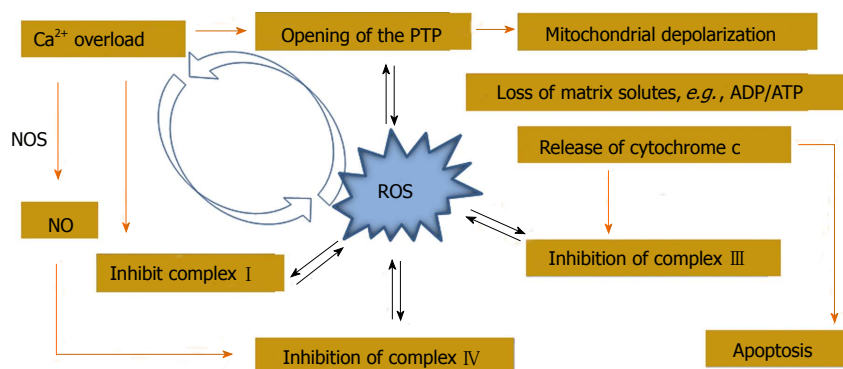


Figure 4 Crosstalk between mitochondrial Ca^{2+} handling and reactive oxygen species generation. PTP: Permeability transition pore; NOS: Nitric oxide synthase; ADP: Adenosine diphosphate; ATP: Adenosine triphosphate.

cell viability during oxygen-glucose deprivation. The protective effects of TRAP1 against oxidative stress-induced cell death can be ascribed to translocation of cytosolic serine/threonine protein kinase, PTEN-induced putative kinase 1, to mitochondria and phosphorylation of TRAP1 that will prevent the release of cytochrome c and thus preserves MPT. TRAP1 expression is found to be elevated in the cardiomyocytes during hypoxia. However, the excess production of ROS in reperfusion/ischemic injury can inhibit the TRAP1 mediated protection that eventually results in the death of cardiomyocytes^[28]. Hence, the role of enzymatic and non-enzymatic antioxidants in the mitochondria has been inevitable to protect the mitochondrial damage. Various mitochondrial antioxidants are useful in alleviating the oxidative stress and are depicted in Figure 2.

The first line of defense against ROS-mediated cardiac injury comprises several antioxidant enzymes, including Mn-superoxide dismutase (MnSOD) and glutathione peroxidase (mtGPx). Among these, mtGPx is an essential enzyme that performs several vital functions. Experimental studies reported the declined cardiac mitochondrial antioxidants, such as activity of Mn-SOD, mtGPx and level of reduced glutathione (GSH) in the myocardium of the aged as well as MI-induced rats^[10]. Besides, the activities of the respiratory chain complexes I-IV and Krebs cycle dehydrogenases also declined^[9]. Several dietary supplements, including the mitochondrial cofactor and antioxidant lipoic acid (LA), can increase the endogenous antioxidants as well as mitochondrial bioenergetics^[6]. Overexpression of the genes for peroxiredoxin-3, a mitochondrial antioxidant, or mitochondrial transcription factor A (TFAM) could ameliorate the decline in mtDNA copy number in failing hearts^[28]. Overexpression of TFAM may protect mtDNA from damage by direct binding and stabilizing of mtDNA. Similarly, overexpression of mtGPx inhibit the development of left ventricular remodeling and failure after MI^[29].

Co-enzyme Q10 (CoQ₁₀) and L-carnitine can be considered to be a safe adjunct to standard therapies in CVD^[30]. CoQ₁₀ is an endogenous compound found in the inner mitochondrial membrane that is essential for electron transport in the ETC and thus for the production of ATP. In addition to its role in bioenergetics,

CoQ₁₀ is demonstrated to be an inhibitor of thrombus formation and able to reduce ROS in mitochondria. Both pre-clinical and clinical studies have shown moderately beneficial effects of CoQ₁₀ in reducing blood pressure, blood glucose and myocardial damage^[31]. Besides the application of CoQ₁₀ in CVD, its use against the adverse effect of drugs, mainly statins, in the intervention of CVD has recently attracted attention. Nevertheless, the antioxidant property of statins^[32,33] can block the endogenous biosynthesis of CoQ₁₀ required for the ETC, resulting in cardiomyopathy and muscle pain^[34]. CoQ₁₀ supplementation (100 mg/d) for 30 d has been found to decrease the muscle pain associated with statin treatment^[35]. In another study, fifty consecutive new patients discontinued 28 mo of statin therapy due to side effects and began CoQ₁₀ supplementation at an average of 240 mg/d^[36] and were followed for an average of 22 mo (84% for more than 12 mo). The prevalence of fatigue from 84% on the initial visit decreased to 16%, the rate of myalgia from 64% to 6%, dyspnea from 58% to 12%, memory loss from 8% to 4% and peripheral neuropathy from 10% to 2%. Moreover, statin-induced cardiomyopathy was found to be reversed with the combination of statin discontinuation and supplementation with CoQ₁₀.

L-carnitine therapy in HF patients (2 g/d, orally) showed improved survival^[37]. A recent study in patients with mild diastolic HF treated with L-carnitine (1.5 g/d, *p.o* for 3 mo) showed improvement in diastolic function^[38]. Therapy with L-carnitine 9 g/d, intravenously for 5 d followed by 6 g/d orally for 12 mo along with the standard medical therapy may limit the adverse effects of acute MI on the heart muscle^[39,40]. Tolerance to exercise was significantly improved in patients with higher left ventricular ejection fraction volume (greater than 30%) when treated with the propionyl-L-carnitine adjunct to appropriate medical therapy^[41].

Carvedilol, both the beta blocker (β_1 , β_2) and alpha blocker (α_1), is indicated in the management of congestive HF. It is used as an adjunct to conventional treatments with its effect probably mediated through the potent antioxidant and anti-apoptotic activities^[42]. The Japanese Diastolic Heart Failure Study has recently suggested the beneficial effects of standard dose prescriptions of carvedilol (> 7.5 mg/d) in HF without affecting

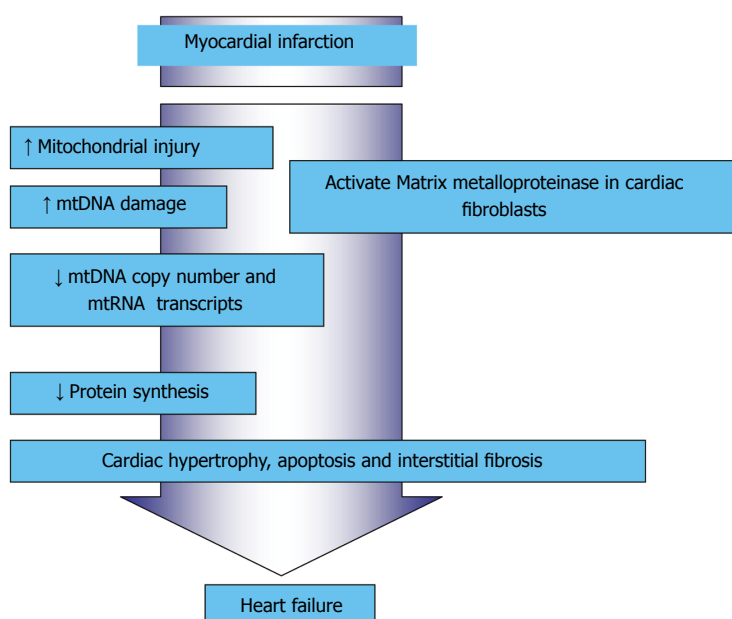


Figure 5 Myocardial infarction-induced mitochondrial damage and dysfunction that resulted in heart failure.

the ejection fraction^[43]. An ACE inhibitor, captopril, was also shown to increase the mitochondrial content in the hearts of dogs following coronary ligation^[44], suggesting that some of its beneficial effects may be due to the stimulation of mitochondrial biogenesis^[45]. However, many extensive clinical trials using conventional antioxidants such as Vitamin E or Vitamin C yielded disappointing results^[46,47]. According to Murphy and Smith^[48], a possible explanation for this may be that the antioxidants are distributed widely in the body with only a small fraction being taken up by mitochondria. Therefore, targeting biologically active molecules to mitochondria will open up avenues for manipulating mitochondrial functions.

FUTURE PERSPECTIVES OF MITOCHONDRIAL PHARMACEUTICS IN CARDIOVASCULAR DISEASES

The increase of mitochondrial concentrations of antioxidant drugs by selective targeting mitochondria should be a practical approach for a wide range of human diseases. Mitochondria-targeted antioxidants have been developed as pharmaceuticals and have been shown to be safe and effective in phase II clinical trials. Various antioxidant molecules targeting mitochondria in cardiomyocytes are given in Table 1. In general, attempts to achieve cell protection using antioxidants have been successfully undertaken with two free radical scavengers, such as 4-hydroxy-2,2,6,6-tetramethylpiperidin-N-oxide and Salen-Mn(III) complex of o-vanillin (EUK-134). Inorganic MnSOD mimetics, such as EUK-8 and EUK-134, possess antioxidant properties of both MnSOD and catalase and have been successfully synthesized and partially tested in terms of their antioxidant and anti-apoptotic properties that appear to be effective in the heart^[49]. The mitochondria-targeted version of vitamin E protected mitochondria from oxidative damage induced by iron/ascorbate far

more effectively than vitamin E itself^[50].

The GSH pool in mitochondria, approximately 15% of total cellular GSH, is found to be reduced during oxidative stress. Choline esters of GSH and N-acetyl-L-cysteine were prepared as mitochondria-targeted antioxidants^[51]. However, *in vivo* data are not available to support their efficacy. Recently, many trials have been conducted in which cationic molecules are targeted using the negative membrane potential of the inner membrane as a promising approach in this field. Triphenylphosphonium (TPP) cation is one among such molecules that are conjugated to a range of antioxidants. Antioxidants ligated with TPP, such as vitamin E^[50], LA^[52], plastoquinone^[53] and mitochondrial CoQ₁₀ (MitoQ)^[54], have been experimentally confirmed to be effective in ameliorating mitochondrial oxidative stress in CVD. TPP, like pentaaza macrocyclic Mn(II) superoxide dismutase (SOD) mimetic, MitoSOD, is found to be very effective in selectively protecting mitochondria from damage^[55].

Adlam *et al.*^[54] reported that the myocardium of the rat administered with MitoQ can render protection against heart dysfunction, tissue damage and mitochondrial dysfunction induced from ischemia-reperfusion injury. It can be given either as *iv* or orally without toxicity. Graham *et al.*^[56] showed that MitoQ protects the development of hypertension, improves endothelial functions and reduces cardiac hypertrophy in young hypertensive rats. MitoQ is also a promising, novel strategy for preserving vascular endothelial function with advancing age and can prevent age-related CVD in mice^[57]. However, MitoQ was not useful in protecting oxidative damage to cardiolipin, accumulation of protein carbonyls, activity of mitochondrial respiratory complexes, mtDNA copy number, or damage to mtDNA^[58-60].

Another critical molecule in this field is a synthetic peptide called Szeto-Schiller (SS) - peptides, synthesized from basic and aromatic amino acids. SS-peptides comprised four alternating aromatic/basic D-amino acids in

Table 1 Mitochondria-targeted antioxidants

Sl no.	Antioxidants
1	4-hydroxy-2,2,6,6-tetramethylpiperidin-N-oxide (TEMPOL)
2	Salen-Mn(III) complex of o-vanillin (EUK-8, EUK-134)
3	Choline esters of glutathione and N-acetyl-L-cysteine
4	Triphenylphosphonium ligated vitamin E, lipoic acid, plastoquinone, Mito SOD and Mito CoQ ₁₀
5	SS-peptides (SS-02 and SS-31)

SS: Szeto-Schiller.

the first or second position with three positive charges at physiological pH. SS-02 and SS-31 were shown to be protective against cardiac ischemia-reperfusion injury when administered on reperfusion by *iv*, *ip* or subcutaneously^[61]. Pre-ischemic intraperitoneal administration of these peptides to rats significantly reduced infarct size^[62]. SS-02 has more efficacy as the free radical scavenger than SS-31. The uptake into tissues or metabolism of these peptides has not yet been thoroughly reported. However, studies with isolated mitochondria showed that despite the cationic nature, these peptides were found to predominantly target the IMM rather than the mitochondrial matrix^[63]. SS-31 is currently in clinical trials for ischemia-reperfusion injury and protects mitochondrial cristae by interacting with cardiolipin on the IMM. SS peptides scavenge H₂O₂ and peroxynitrite inhibits lipid peroxidation. They can also inhibit the decline of MPT and cytochrome c release, thus preventing oxidant-induced cell death^[64]. Although the delivery of antioxidants may protect mitochondria from oxidative stress caused by a variety of insults, the area of mitochondria-specific delivery of drugs is still in its infancy. Among the molecules studied in CVD, clinical trials on CoQ₁₀ have found that it can be considered a safe adjunct to conventional therapies in CVD. Despite the beneficial effect of CoQ₁₀, given alone or in addition to conventional therapies in hypertension and in HF, less extensive evidence in IHD has been found^[65]. The present findings demonstrate that mitochondrial damage plays a prominent role in HF following MI and further research into the role of mitochondria-targeted agents to prevent the HF is compulsory.

CONCLUSION

Mitochondrial dysfunction plays a key role in the pathogenesis of ischemia and reperfusion injury and cardiomyopathy. Mutations in mt DNA and abnormalities in mitochondrial function are associated with common forms of cardiac diseases. Despite the promising mitochondria-targeted drugs that are emerging from the laboratory, very few have successfully completed clinical trials. Antioxidants ligated with TPP, such as vitamin E, lipoic acid, plastoquinone, MnSOD and mitochondrial CoQ₁₀, have been experimentally determined as effective in ameliorating the mitochondrial oxidative stress associated with CVD. Among the molecules targeting mitochondria, MitoQ provides a novel approach to attenuate oxidative

damage with the potential to become a new therapeutic intervention in humans. However, there are insufficient data from well designed randomized trials to issue a general recommendation for people to take antioxidant supplements in order to prevent heart disease. Since mitochondrial damage is central to the pathophysiology of HF, various approaches used to target antioxidant compounds at mitochondria should be explored in the development for the treatment of HF. A great deal of future research will be needed before mitochondria-directed therapies are made available for the prevention and treatment of CVD.

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Perioperative clinical variables and long-term survival following vascular surgery

Santiago Garcia, Edward O McFalls

Santiago Garcia, Minneapolis VA Healthcare System, University of Minnesota, MN 55417, United States

Edward O McFalls, Division of Cardiology (111C), VA Medical Center, Minneapolis VA Healthcare System, Professor of Medicine, University of Minnesota, MN 55417, United States

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Correspondence to: Edward O McFalls, MD, PhD, Division of Cardiology (111C), VA Medical Center, Minneapolis VA Healthcare System, Professor of Medicine, University of Minnesota, 1 Veterans Drive, Minneapolis, MN 55417, United States. mcfal001@umn.edu

Telephone: +1-612-4673664 Fax: +1-612-7275668

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Abstract

Cardiovascular disease is the leading cause of death in patients with peripheral arterial disease (PAD). Coronary artery disease (CAD) is highly prevalent, and often times coexist, in patients with PAD. The management of patients with PAD that requires a high-risk vascular surgical procedure for intermittent claudication, critical limb ischemia or expanding abdominal aortic aneurysm requires risk stratification with the revised cardiac risk index, optimization of medical therapies, and limited use of cardiac imaging prior to surgery. Preventive revascularization in patients with stable CAD, with the sole intention to mitigate the risk of cardiac complications in the peri-operative period, is not effective and may be associated with significant bleeding and thrombotic risks, in particular if stents are used. A strategy of universal use of cardiac troponins in the perioperative period for active surveillance of myocardial ischemia may be more reasonable and cost-effective than the current standard of care of widespread use of cardiac imaging prior to high-risk surgery. An elevated cardiac

troponin after vascular surgery is predictive of long-term mortality risk. Medical therapies such as aspirin and statins are recommended for patients with post-operative myocardial ischemia. Ongoing trials are assessing the role of novel anticoagulants. Additional research is needed to define the role of cardiac imaging and invasive angiography in this population.

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Key words: Peripheral arterial disease; Myocardial infarction; Coronary artery disease; Prognosis; Coronary revascularization

Core tip: Patients with advanced peripheral arterial disease who need vascular surgery have a high prevalence of coronary atherosclerosis and are at increased risk of perioperative myocardial infarction. Coronary revascularization prior to the vascular operation is not an effective intervention to mitigate this risk. A strategy of widespread use of cardiac troponins in the perioperative period is recommended to detect perioperative ischemic events associated with a long-term mortality risk. The selective use of medical interventions, cardiac imaging and coronary angiography in this population deserves further study.

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INTRODUCTION

The approach to patients with peripheral arterial disease (PAD) is best appreciated in the broader context of the epidemiology of the disease, risk factors, and surgical and endovascular interventions to improve symptoms, pre-

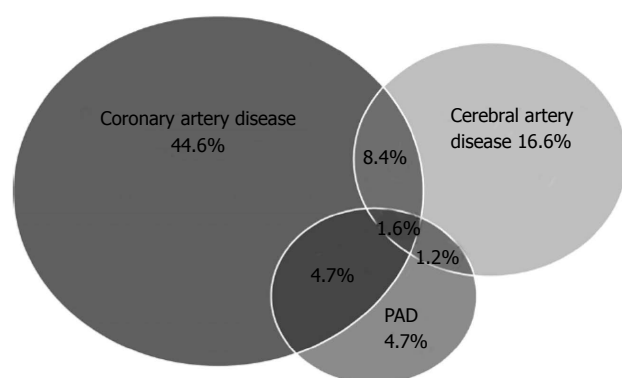


Figure 1 Data from the Reduction of Atherothrombosis for Continued Health registry. Approximately 50% of patients with peripheral arterial disease (PAD) and polyvascular disease have concomitant coronary artery disease. Reproduced with permission from society for vascular surgery.

serve limb viability or prevent aneurismal rupture.

Peripheral arterial disease (PAD) and coronary artery disease (CAD) often coexist in the same patient and share a common risk factor profile, pathophysiology, and array of therapeutic interventions^[1-3]. Cardiovascular disease is the leading cause of death in patients with PAD, responsible for about two of every three deaths^[4].

Vascular surgery is considered a high-risk operation with one in four patients experiencing a peri-operative myocardial infarction (PMI), which is associated with increased long-term mortality^[5,6]. Identifying the clinical variables associated with increased risk of PMI prior to surgery as well as defining the best strategy for surveillance of PMI after high-risk surgery are of critical importance in clinical practice to mitigate risk and improve outcomes.

Definition of PAD

The definition of PAD is based on a resting ankle-brachial index (ABI) of ≤ 0.90 ^[1]. Noticeably, the presence of symptoms is not required to diagnose PAD. For every patient with symptoms of PAD there are 4 with no symptoms as defined by ABI or duplex ultrasonography^[7]. Screening for PAD is therefore recommended to detect the disease in individuals with a high pre-test probability. In the PARTNERS (PAD Awareness, Risk, and Treatment: New Resources for Survival) study the prevalence of PAD defined by ABI was 29%^[8]. Therefore, current ACC/AHA guidelines recommend screening for PAD in patients aged ≥ 70 or 50-69 years with a risk factor for vascular disease^[1]. Intermittent claudication (IC) is the most common presenting symptom of symptomatic PAD. IC is characterized by leg pain (muscular pain) with activity that is relieved by physical rest. Claudication tends to occur one anatomical level below the arterial level of obstruction or occlusion. For example a patient with superficial femoral artery (SFA) occlusion will likely have calf symptoms. The prevalence of IC in the general population is low but increases significantly with age so that in patients aged 60 or older is about 6%^[9].

Risk factors for PAD

The risk factors for developing PAD and CAD show significant overlap and include male gender, age, hypertension, hyperlipidemia, renal insufficiency, black race, and more importantly diabetes mellitus (DM) and smoking, both of which have odds ratios (ORs) over 3 for symptomatic PAD^[2,10-14]. Likewise, diabetics and smokers have a 3 to 4-fold increase in the risk of developing critical limb ischemia and amputations^[2,12].

POLYVASCULAR DISEASE

The prevalence of CAD in patients with PAD depends on the setting and the sensitivity of the method used to identify occult CAD. In the REACH (Reduction of Atherothrombosis for Continued Health) outpatient registry (Figure 1), 50% of patients with PAD and polyvascular disease had coexistent CAD^[3]. In a landmark angiographic study of 1000 patients undergoing coronary angiography prior to vascular surgery conducted at the Cleveland Clinic by Hertzner *et al.*^[15] only 8% had normal coronary arteries prior to surgery, 2/3 had severe CAD, 10% had inoperable CAD and 18% had moderate CAD.

The annual rate of major adverse cardiovascular events (MACE) (myocardial infarction, stroke, and vascular death) in patients with PAD is 5%-7%^[1,2]. Critical limb ischemia (CLI) patients have 20% mortality only in the first year after initial presentation. CAD is responsible for 40%-60% of deaths among patients with PAD while cerebral arterial disease accounts for another 10%-20% of deaths^[1,2,4]. The severity of PAD, as quantified by ABI, correlates with the risk of MACE so that for every 0.10 decrease in ABI there is a corresponding 10% increase in MACE^[16]. There is a strong association between MACE and ABI ≤ 0.60 in patients with diabetes^[16] (Figure 2).

CARDIAC RISK STRATIFICATION PRIOR TO VASCULAR SURGERY

Variables to assess prior to a vascular operation include the type of operation (open *vs* endovascular), the risk of concomitant CAD and the functional status of the patient^[17]. Open abdominal aneurismal repair with cross-clamp of the aorta and non-elective operations carry the highest risk of cardiovascular complications^[18] in part due to the hemodynamic stress of the surgery, CAD burden, and the acuity of the condition that often hampers the ability to start preoperative interventions to mitigate cardiac risk.

Evaluating the functional status of subjects undergoing vascular surgery is an important step in assessing if a patient can tolerate the hemodynamic stress of a prolonged surgery. If a patient is unable to achieve a metabolic demand of 4-METS, which is a level compatible with routine activities of daily living, the risk of surgical complications increases and additional testing may be warranted. Stress imaging testing, usually with pharma-

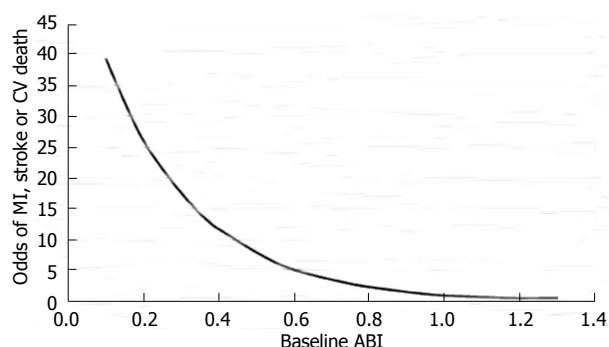


Figure 2 Odds of a major cardiovascular event according to baseline ankle-brachial index in patients with diabetes mellitus. Reproduced with permission from Mehler *et al.*^[16]. MI: Myocardial infarction; CV: Cardiovascular; ABI: Ankle-brachial index.

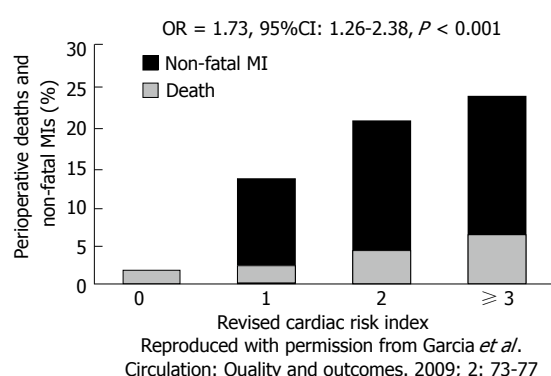


Figure 3 Outcomes at 30 d following vascular surgery according to number of risks as enumerated in the revised cardiac risk index. MI: Myocardial infarction.

cological agents such as adenosine or dobutamine, has been recommended prior to high-risk vascular surgery in patients with functional capacity < 4 METS^[17]. The presence of large or multiple ischemic segments or transient ischemic dilatation of the left ventricle may indicate either multivessel or left main CAD. These findings are considered high risk and are associated with an increased risk of perioperative cardiac complications and reduced long-term survival^[19]. Coronary angiography is recommended to patients that have high-risk findings on non-invasive imaging, as certain angiographic subsets (*i.e.*, left main CAD) derive a long-term benefit from revascularization^[20]. An initial approach that combines clinical and stress-imaging variables is cost-effective^[21].

The Revised Cardiac Risk Index (RCRI) is a risk score comprised of six clinical variables (Table 1) that has been validated in a general surgery population as a tool to predict the risk of cardiac adverse events at 30 d^[22]. A RCRI ≥ 3 is associated with > 5% risk of a serious cardiac complication in the postoperative period. However, in vascular surgery the RCRI tends to underestimate the risk of cardiac complications. In the Coronary Artery Revascularization Prophylaxis (CARP) trial a RCRI > 1 was predictive of a 10% risk of MI or death at 30 d in the preoperative revascularization (PR) group and 15% in the medical arm (Figure 3)^[23].

Table 1 The Revised Cardiac Risk Index is comprised of 6 clinical variables that receive 1 point if present and 0 if absent

High-risk procedures (<i>i.e.</i> , vascular surgery)
History of cerebrovascular disease
History of coronary artery disease
History of congestive heart failure
Creatinine > 2.0 mg%
Diabetes (insulin-dependent)

A score ≥ 3 predicts a 10% risk of serious cardiac complications after non-cardiac surgery.

PREOPERATIVE CORONARY REVASCULARIZATION

The CARP Trial was a randomized, multisite VA study designed to assess the role of PR in patients with CAD undergoing elective vascular surgery^[24]. A total of 510 patients were enrolled and randomized to either PR or no PR prior to elective vascular surgery. Indications for surgery included an expanding AAA in 33% of patients and arterial occlusive disease of the lower limbs in 67%. The index revascularization procedure consisted of percutaneous coronary intervention (PCI) in 59% and coronary artery bypass graft (CABG) surgery in 41% of patients. At 2.7 years, mortality in the PR group was 22% and in the no PR group was 23% ($P = 0.92$; RR = 0.98, 95%CI: 0.70-1.37) (Figure 4). Similarly, no difference in outcomes was seen within 30-d, mortality was 3.1% in the PR group and 3.4% in the no PR group ($P = 0.87$) and a MI occurred in 11.6% of the PR group and 14.3% of the no PR group ($P = 0.37$). The main conclusion of the CARP study is that preoperative coronary artery revascularization prior to vascular surgery does not result in better short- or long-term clinical outcomes in patients with stable CAD.

The pilot Dutch Echocardiographic Cardiac Risk Evaluation Applying Stress Echo (DECREASE) - V study randomized 101 patients with stress-induced ischemia and multivessel or left main CAD to PR or no PR prior to high-risk vascular surgery^[25]. At 1-year, the composite of non-fatal myocardial infarction and mortality between groups (49% *vs* 44%, $P = 0.48$) was no different. Taken together these data do not support a strategy of PR prior to elective vascular surgery in patients with stable CAD.

PERIOPERATIVE MYOCARDIAL INFARCTION

Definition and predictors

The Third Universal Definition of myocardial infarction (MI) proposed by the ESC/ACCF/AHA/WHF task force requires a rise and fall of cardiac biomarkers, preferably troponins, with at least one value above the 99th percentile of the upper reference limit (URL) coupled with a clinical correlate of ischemia such as ischemic

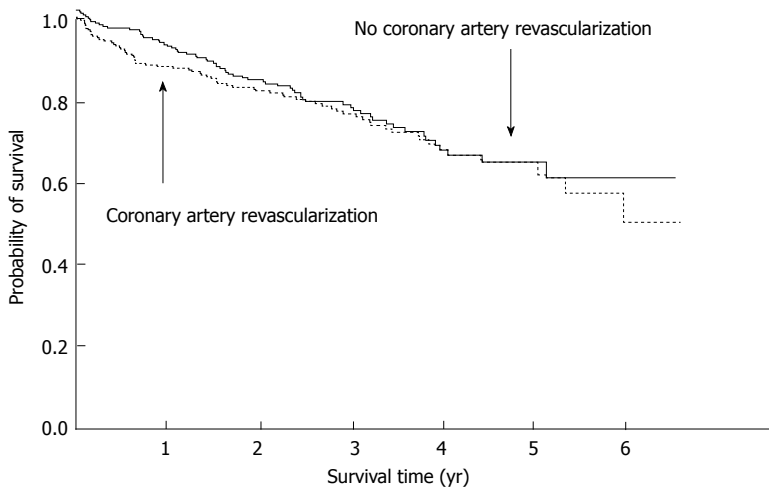


Figure 4 Primary outcome of the coronary artery revascularization prophylaxis trial: Overall survival at 2.7 yr was no different between groups (22 % vs 23%).

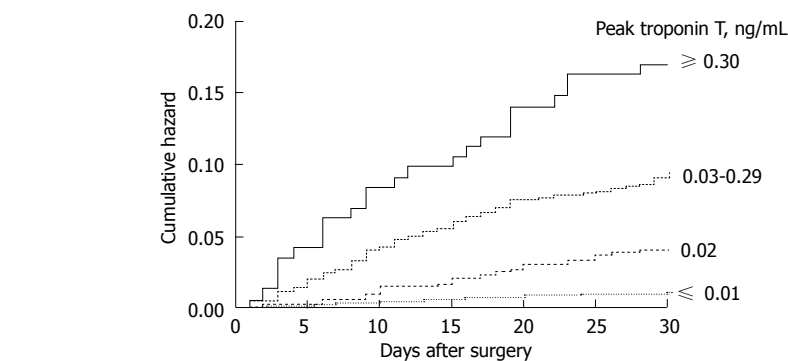


Figure 5 The vascular events in Noncardiac Surgery Patients Cohort Evaluation Study. Peak troponin T values (TnT) of 0.02 ng/mL were associated with increased (4%) risk of death at 30 d relative to TnT levels < 0.01. Reproduced with permission from Devereaux *et al*.^[28]

NO. at risk	0	5	10	15	20	25	30
Peak troponin T, ng/mL							
≥ 0.30	142	136	129	127	121	118	117
0.03-0.29	1121	1103	1075	1058	1036	1030	1018
0.02	494	492	489	485	480	477	473
≤ 0.01	13376	13348	13300	13271	13250	13230	13209

symptoms, electrocardiographic ischemic changes, or imaging criteria of new loss of previously viable myocardium^[26]. However, owing to the effects of anesthesia, and other factors such as widespread use of narcotics, the vast majority of perioperative ischemic events are clinically silent. In the Perioperative ischemic evaluation (POISE) trial 65% of patients with a perioperative ischemic event did not experience ischemic symptoms^[27]. The risk of death at 30 d was 9.7% in patients with a symptomatic MI and 12.5% in patients with an asymptomatic MI. Thus, the universal definition of MI may not be as sensitive in the perioperative period to detect ischemic events that are associated with poor intermediate- and long-term outcomes. An isolated peak cardiac biomarker elevation (preferably troponins) above the 99th URL, with or without a correlate of ischemia, may be the most sensitive tool to detect perioperative ischemic events that are clinically important. In the Vascular Events in Noncardiac Surgery Patients Cohort Evaluation (VISION) registry^[28], a peak postoperative troponin T (TnT) measured within the first 3 d after surgery was the strongest predictor of 30-d mortality and explained 41.8% of the deaths in population attributable risk analysis (Figure 5). A peak TnT of 0.02 ng/mL was associated with a 4% risk of death at 30 d^[28].

Preoperative clinical variables that predicted 30-d

mortality risk and were retained in the model that included peak TnT values included: age > 65, recent history of high-risk CAD, peripheral arterial disease, history of stroke, chronic obstructive pulmonary disease (COPD), cancer, urgent/emergency surgery, and major general or neurosurgical procedures. Of note, major vascular surgery and diabetes were not predictive of 30-d mortality in the model that included TnT^[28].

In a cohort of 377 patients included in the CARP trial in whom cardiac troponin I was measured and analyzed by a core lab after the vascular surgery the proportion of patients with a perioperative myocardial infarction was 26%. Independent predictors of an MI included: age > 70 (OR = 1.84; 95%CI: 1.14-2.98; *P* = 0.01), abdominal aortic surgery (OR = 1.82; 95%CI: 1.09-3.03; *P* = 0.02), diabetes (OR = 1.86; 95%CI: 1.11-3.11; *P* = 0.02), angina (OR = 1.67; 95%CI: 1.03-2.64; *P* = 0.04), and baseline ST-T wave abnormalities (OR = 1.62; 95%CI: 1.00-2.6; *P* = 0.05)^[29].

Pathophysiology

Clinical, angiographic, and pathological studies have shed light into the mechanisms underlying postoperative ischemic events^[30-33]. Most of these events are caused by a mismatch between O₂ supply and demand, usually with severe CAD in the background that is unmasked by

the stress of the surgery. Landesberg *et al*^[30] showed that ST-segment depression related to rapid heart rates is common in the perioperative period and predictive of long-term mortality. The duration of ST-segment depression and peak catecholamine levels after surgery are associated with infarct size. Chronic total occlusions (CTOs) are common in patients with a perioperative ischemic event or cardiac death (81%) relative to only 29% in patients without ischemic complications after surgery^[31]. Two pathological studies reported conflicting data on the incidence of plaque rupture after fatal postoperative MI^[32-33]. Dawood *et al*^[32] described evidence of plaque rupture in only 7% of patients (42 autopsies). Conversely, a higher incidence of plaque rupture (46%) was described by Cohen *et al*^[33]. Differences in timing of the autopsy relative to the time of the MI may account for some of the discrepancies in the data.

Management of perioperative myocardial infarction

Data from randomized clinical trials are lacking to guide therapy in the postoperative period. Small studies have shown that interventions aimed at improving oxygen delivery and minimizing myocardial oxygen consumption are beneficial in this setting^[34]. The main goal of therapy is to preserve coronary perfusion pressure during diastole. This is best achieved with judicious utilization of beta-blockers, analgesia, and fluid administration with the intention to avoid tachycardia and hypotension. In the POISE trial for every 10-beats/min increase in heart rate there was a 31% relative increase in the odds of perioperative MI^[27]. Current guidelines recommend aggressive blood pressure control in patients with PAD, in particular in patients with diabetes and/or chronic kidney disease (goal < 130/80 mmHg)^[35]. In the HOPE (Heart Outcomes Prevention Evaluation) trial ramipril 10 mg was associated with a 22% reduction in cardiovascular events and is currently recommended for high-risk patients, including those with PAD^[36].

Statins contribute to plaque stabilization by decreasing circulating levels of inflammatory cytokines and reactive oxygen species while increasing expression of nitric oxide synthase^[37]. Additionally, evidence from randomized clinical trials and observational studies support its use in clinical practice. In the DECREASE-III study a 53% reduction in CV death and myocardial infarction was seen with high-dose fluvastatin in patients undergoing vascular surgery^[38]. In another trial of 100 patients randomly assigned to 20 mg of atorvastatin or placebo prior to vascular surgery, the use of statins was associated with a significant reduction in cardiac events, from 26% to 8% at 6 mo^[39]. An observational study of 164 veterans undergoing vascular surgery at our medical center demonstrated that utilization of statin drugs was associated with a reduction in long-term mortality^[5]. Guidelines recommend the use of statins in patients with peripheral arterial disease to reduce cardiovascular events^[2].

Owing to concerns for bleeding after non-cardiac surgery, the use of medical therapies and interventional

strategies commonly used to treat spontaneous MIs such as antiplatelet agents, anticoagulants and invasive coronary angiography are rarely used in this setting and have not been extensively studied in clinical trials. The management of myocardial infarction After NonCardiac surgery (MANAGE) trial (NCT01661101) will be the first study to randomize patients ($n = 3200$) with a PMI after noncardiac surgery to dabigatran or placebo. The primary end point is the occurrence of a major vascular complication (vascular mortality, nonfatal MI, nonfatal stroke, and pulmonary embolism). The trial plans to complete enrollment in November 2015.

FUTURE DIRECTIONS

Another strategy for prevention of myocardial ischemia during surgery is ischemic preconditioning, which describes the protection afforded by application of non-lethal episodes of myocardial ischemia prior to the index ischemic event^[40,41]. The Cardiac Remote Ischemic Preconditioning Prior to Elective Vascular Surgery (CRIPES, NCT: 01558596) was designed to determine the feasibility and safety of using remote ischemic preconditioning (RIPC) prior to vascular surgery, and to obtain preliminary estimates of its effects on detectable postsurgical increases in cardiac troponin I^[42]. A similar strategy of RIPC has been evaluated prior to coronary angioplasty^[43] and coronary artery bypass surgery^[44] with positive initial results.

CONCLUSION

Patients with PAD in need of elective vascular surgery have a high prevalence of coronary atherosclerosis and are at increased risk of perioperative myocardial infarction. Coronary revascularization prior to the vascular operation is not an effective intervention to mitigate this risk. A strategy of widespread use of cardiac troponins in the perioperative period is recommended to detect perioperative ischemic events associated with a long-term mortality risk. The selective use of medical interventions, cardiac imaging and coronary angiography in this population deserves further study.

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Quantitative assessment of myocardial blush grade in patients with coronary artery disease and in cardiac transplant recipients

Nina Patricia Hofmann, Hartmut Dickhaus, Hugo A Katus, Grigorios Korosoglou

Nina Patricia Hofmann, Hugo A Katus, Grigorios Korosoglou, Department of Cardiology, University of Heidelberg, 69120 Heidelberg, Germany

Hartmut Dickhaus, Department of Medical Informatics, University of Heidelberg, 69120 Heidelberg, Germany

Author contributions: All the authors together contributed to this paper.

Correspondence to: Nina Patricia Hofmann, MD, Department of Cardiology, University of Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany. nina.hofmann@med.uni-heidelberg.de

Telephone: +49-6221-5637487

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Abstract

Quantitative assessment of myocardial perfusion by myocardial blush grade (MBG) is an angiographic computer-assisted method to assess myocardial tissue-level reperfusion in patients with acute coronary syndromes and microvascular integrity in heart transplant recipients with suspected cardiac allograft vasculopathy. This review describes the ability of quantitative MBG as a simple, fast and cost effective modality for the prompt diagnosis of impaired microvascular integrity during routine cardiac catheterization. Herein, we summarize the existing evidence, its usefulness in the clinical routine, and compare this method to other techniques which can be used for the assessment of myocardial perfusion.

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Key words: Quantitative myocardial blush grade; Prognosis; Heart transplantation; Coronary artery disease

Core tip: In this article, we highlight the ability of

quantitative myocardial blush grade for the assessment of microvascular integrity in patients with acute coronary syndromes (ACS) and heart transplant (HT) recipients with cardiac allograft vasculopathy (CAV). Using an, in the meanwhile well-established, computational algorithm, a prompt diagnosis can be made in the catheterization lab, which can identify patients with ACS and increased risk for myocardial remodelling and congestive heart failure in the long-term. In addition, this computational algorithm can identify HT recipients with increased risk for CAV and adverse cardiovascular outcomes.

Hofmann NP, Dickhaus H, Katus HA, Korosoglou G. Quantitative assessment of myocardial blush grade in patients with coronary artery disease and in cardiac transplant recipients. *World J Cardiol* 2014; 6(10): 1108-1112 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i10/1108.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i10.1108>

INTRODUCTION

Impaired myocardial perfusion by either epicardial coronary artery disease (CAD) or small vessel disease is a common challenge in cardiology worldwide. Both CAD and cardiac allograft vasculopathy (CAV) in heart transplant (HT) recipients significantly influence mortality in such patient cohorts. Considering the vast amount of health care costs for post-rehabilitation support after myocardial infarction^[1] and HT^[2], preventive medical care should primarily focus on the early detection of cardiac pathology and risk stratification of such patients. Quantitative assessment of epicardial and microvascular integrity can aid tailoring pharmacologic therapy of patients identified at high risk for future events, which may ultimately improve clinical outcomes. We therefore

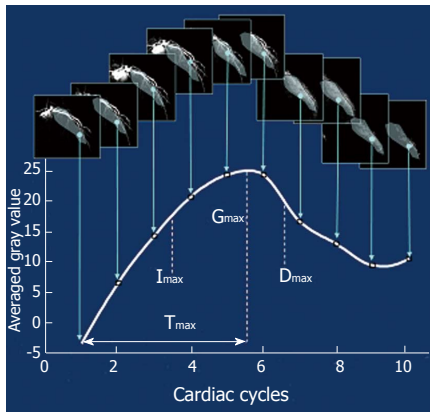


Figure 1 Digital subtraction images of a myocardial region of interest illustrate the temporal distribution of grey level rise and venous washout of contrast agent during a coronary catheter image sequence.

previously developed a computer-assisted program for the analysis of microvascular integrity in patients undergoing cardiac catheterization either during acute myocardial infarction for reperfusion of the infarcted tissue^[3,4] or during surveillance coronary angiography in cardiac transplant recipients^[5,6]. Furthermore, such measures of perfusion can also be applied in patients undergoing fractional flow reserve analysis (FFR) for the assessment of the functional significance of coronary lesions of moderate severity^[7].

METHODOLOGICAL APPROACH WITH OUR MYOCARDIAL BLUSH GRADE ALGORITHM

We previously introduced a computer-based algorithm for the quantification of MBG in patients with first time acute myocardial infarction^[3]. This method aimed at the objective assessment of reperfused myocardial tissue and the estimation of infarct size and functional recovery of the myocardium at risk. This method is based on conventional cine angiographic films. In order to achieve maximal quality of the digital subtraction angiography images, the sequence is synchronized with the baseline electrocardiogram (ECG). The spatio-temporal spread of blood, or the so-called MBG, through epicardial vessels and then to the microvasculature and the myocardium, indicated by dye injection, represents a characteristic pattern for the myocardial perfusion. This dynamic temporal pattern is characterized by typical features as the maximal value of MBG intensity and the increase and decrease velocity which correspond to the different phases of flooding in and washout. Regions of interest are positioned in the distal part of each coronary artery in order to measure the plateau of mean grey level pixel intensity (G_{max} which is measured on a standard gray scale of 0 to 255) as well as the time to maximal intensity rise (T_{max} measured in seconds) (Figure 1). The ratio G_{max}/T_{max} is subsequently computed in each coro-

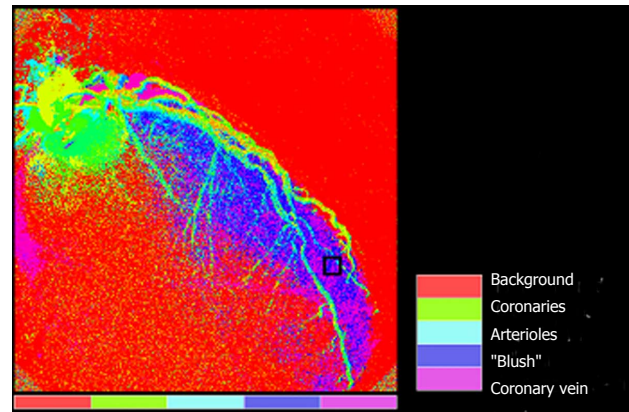


Figure 2 The parametric image shows coloured phases of cardiac perfusion in a combined presentation. The arterial phase is followed by early and late myocardial infusion until the contrast agent arrives in the venous system.

nary vessel. Furthermore, based on the distribution of MBG over time in the epicardial vessels, arterioles and capillaries, parametric quantification can be applied as shown in Figure 2. To allow for quantification of MBG, frames should be recorded long enough in order to allow filling of the coronary veins, and images should be acquired during breath hold in order to avoid artefacts due to movement of the diaphragm. On the basis of 100 different temporal MBG profiles, an algorithm is established which classifies the acquired blush patterns into 4 different grades^[8]. An example of a patient with post-interventional high G_{max}/T_{max} and full functional recovery after first acute non-ST-elevation myocardial infarction (NSTEMI) of the left anterior descending is illustrated in Figure 3.

CURRENT EVIDENCE

In a swine model by Boyle *et al*^[9], the quantitative myocardial blush grades could be assessed automatically and were closely related to established angiographic parameters of myocardial perfusion.

Our first clinical findings showed that quantitative MBG is applicable for the evaluation of microvascular tissue perfusion in patients with ST-elevation myocardial infarction (STEMI), being highly predictive for functional recovery of the myocardium at risk as assessed by echocardiography^[3]. Hereby, multivariate analysis showed that MBG and Troponin T elevation were independent predictors of residual ejection fraction > 50%. Quantitative MBG by G_{max}/T_{max} showed the highest odds ratio and was therefore considered as the most robust variable for the prediction of the primary endpoint.

Furthermore, quantitative MBG was related to infarct transmural and residual ejection fraction by cardiac magnetic resonance (CMR) in both STEMI and NSTEMI patients. This objective information can be acquired during routine cardiac catheterization, immediately after interventional treatment of the infarct related lesion, and can be used for the immediate risk stratification of pa-

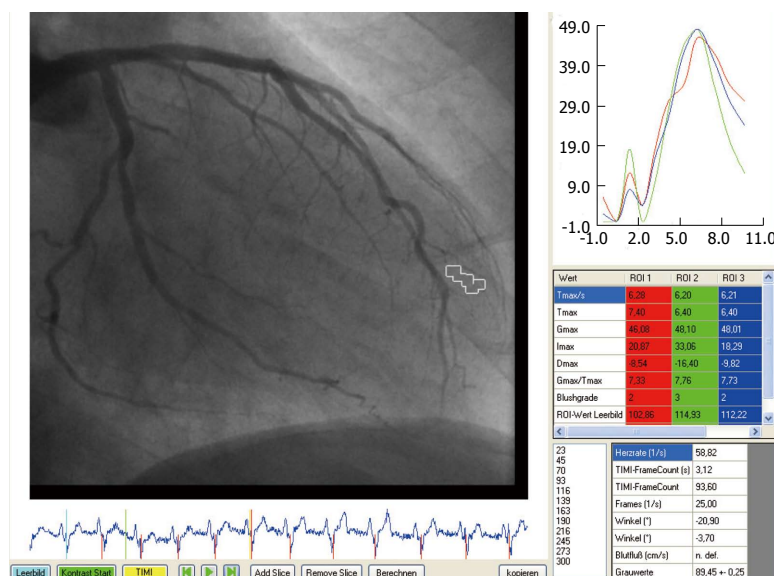


Figure 3 Computer-assisted program illustrating post-interventional high myocardial blush grade after successful left anterior descending revascularization in a non-ST-elevation myocardial infarction patient.

tients with ACS^[4]. Hereby, G_{\max}/T_{\max} was at least as accurate as infarct transmuralty for the prediction of residual ejection fraction. Both clearly surpassed the accuracy of visual MBG.

Besides patients with CAD, we also investigated our computer-assisted program on the growing group of HT recipients. For this purpose, transplanted patients who underwent surveillance cardiac catheterization were subsequently analyzed by CMR, to assess myocardial relaxation and perfusion reserve during adenosine stress. Close correlations were observed between G_{\max}/T_{\max} with perfusion reserve and with mean diastolic strain rates. Visual and quantitative MBG had significantly higher accuracy than stenosis severity on coronary angiograms for the detection of diminished myocardial perfusion. Furthermore, quantitative MBG provided more robust prediction of survival compared to visually estimated blush and to coronary lumen narrowing assessment. Hereby, our findings indicate that quantitative MBG can be performed on coronary angiograms of HT recipients just as well, and may aid the detection of CAV in such individuals with impaired perfusion but with angiographically “normal” coronaries^[5].

Impaired myocardial perfusion in transplanted hearts is closely associated with outcomes. In this regard, G_{\max}/T_{\max} is a simple to acquire and useful surrogate parameter of myocardial perfusion in HT recipients, which can predict cardiac outcomes. G_{\max}/T_{\max} differentiated between patients with high rate of cardiac events compared to those with higher quantitative MBG, who exhibited much better outcomes during a mean follow-up duration of 2.7 years. In addition, close correlations were observed between MBG and perfusion reserve measured by stress magnetic resonance imaging. Quantification of MBG may therefore be useful for the risk stratification of such patients^[6].

Finally, preliminary clinical data indicate that MBG during adenosine infusion can be used to estimate an-

giographic perfusion reserve and is associated with FFR measures and with the myocardial perfusion reserve, assessed by CMR^[7].

COMPARISON TO OTHER INVASIVE AND NON-INVASIVE APPROACHES FOR THE ASSESSMENT OF MYOCARDIAL PERFUSION

So far, different non-invasive and invasive imaging methods have been used for analysis and risk stratification of CAD and CAV patients, as, *e.g.*, myocardial contrast echocardiography (MCE), CMR imaging and angiographic parameters including Thrombolysis in Myocardial Infarction (TIMI) flow grade, TIMI frame count, TIMI myocardial perfusion grade and MBG.

NON-INVASIVE IMAGING COMPARED TO ANGIOGRAPHIC PERFUSION MEASURES

Myocardial contrast echocardiography

Myocardial perfusion and function can be assessed during MCE. This technique provides real-time visualisation of ischemic myocardium in regions of reduced blood flow. Although MCE is a practicable and non-invasive technique, it is limited by observer dependency and technical challenges pending on patients' echogenic windows^[10].

CMR imaging

This is the current reference method for the assessment of cardiac anatomy, perfusion and function, viability and if required metabolism, all within a single examination, non-invasively and without ionizing radiation for the patients. Therefore, our studies mostly compare MBG to either CMR derived ejection fraction, remodelling, infarct size and transmuralty or perfusion reserve index^[4-6,11].

INVASIVE ASSESSMENT OF CARDIAC PERFUSION IN PATIENTS WITH ACS: FROM THE EPICARDIUM TO THE MYOCARDIUM

TIMI flow grades and TIMI frame count

Reperfusion after myocardial infarction or ACS determines clinical outcome^[12]. However, clinical and experimental data indicate that stenosis reduction during percutaneous coronary intervention (PCI) is not always associated with adequate myocardial tissue reperfusion, so that patients with TIMI 3 flow grade after PCI may still exhibit impaired microvascular integrity^[3,13,14]. Thus, epicardial restoration of coronary blood flow is only prerequisite, but not a guarantee for myocardial recovery^[15], the latter being a major predictor of mortality and morbidity in CAD patients.

TIMI myocardial perfusion grade and visual myocardial blush grade

Visually assessed MBG represents a reasonable alternative to TIMI flow grade and TIMI frame count, since it can distinguish between high and low risk constellations. The TIMI myocardial perfusion grade demonstrates a similar method that also considers the dynamic contrast agent washout. Unfortunately, the accuracy of both techniques is limited due to their categorical nature, which is associated with high observer variability, especially with non-expert readers.

Quantitative myocardial blush grade

The “Quantitative Blush Evaluator” (QuBE) from the TAPAS trial, an open-source computer program for quantification of myocardial perfusion, was used on angiograms in patients with acute STEMI^[16]. The QuBE score correlated significantly with visual MBG as well as infarct size and microvascular dysfunction assessed by CMR^[17]. Nevertheless, it has exclusively been used for STEMI patients so far, and further evaluation in other patient cohorts is warranted.

CONCLUSION

Quantitative assessment of MBG can be performed on coronary angiograms of either CAD or CAV patients. In this regard, G_{\max}/T_{\max} is a simple and useful surrogate parameter of microvascular integrity, which can (1) estimate clinical outcome in HT recipients with impaired perfusion reserve but without angiographically evident atherosclerosis and (2) infarct transmural and functional recovery in both STEMI and NSTEMI. These results can be used for tailoring pharmacological treatment and aid early risk stratification in both CAD and CAV patients.

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Neuroticism personality trait is associated with Quality of Life in patients with Chronic Heart Failure

Lampros Samartzis, Stavros Dimopoulos, Christos Manetos, Varvara Agapitou, Athanasios Tasoulis, Eleni Tseliou, Iraklis Pozios, Elisavet Kaldara, John Terrovitis, Serafim Nanas

Lampros Samartzis, St. George's University of London Medical School at University of Nicosia, 2408 Nicosia, Cyprus

Lampros Samartzis, Department of Psychiatry, Nicosia Mental Health Services, Athalassa Psychiatric Hospital, 1432 Nicosia, Cyprus

Lampros Samartzis, Stavros Dimopoulos, Christos Manetos, Varvara Agapitou, Athanasios Tasoulis, Serafim Nanas, 1st Critical Care Medicine Department, Cardiopulmonary Exercise Testing and Rehabilitation Laboratory, "Evgenidio" Hospital, National and Kapodistrian University of Athens, 15128 Athens, Greece
Eleni Tseliou, Iraklis Pozios, Elisavet Kaldara, John Terrovitis, 3rd Cardiology Department, "Laiko" Hospital, National and Kapodistrian University of Athens, 15128 Athens, Greece

Author contributions: Samartzis L, Dimopoulos S and Nanas S designed the experiments; Manetos C, Agapitou V, Tasoulis A, Tseliou E, Pozios I and Kaldara E prepared the experiments; Samartzis L and Dimopoulos S prepared the manuscript; Terrovitis J and Nanas S revised and approved the final version of the manuscript.

Correspondence to: Stavros Dimopoulos, MD, 1st Critical Care Department, Cardiopulmonary Exercise Testing and Rehabilitation Laboratory, "Evgenidio" Hospital, National and Kapodistrian University of Athens, 20, Papadiamantopoulou str, 15128 Athens, Greece. a-icu@med.uoa.gr

Telephone: +30-697-3956974 Fax: +30-210-7242785

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Abstract

AIM: To evaluate Quality of life (QoL) in chronic heart failure (CHF) in relation to Neuroticism personality trait and CHF severity.

METHODS: Thirty six consecutive, outpatients with Chronic Heart Failure (6 females and 30 males, mean age: 54 ± 12 years), with a left ventricular ejection fraction $\leq 45\%$ at optimal medical treatment at the time of inclusion, were asked to answer the Kansas City Cardiomyopathy Questionnaire (KCCQ) for Quality of

Life assessment and the NEO Five-Factor Personality Inventory for personality assessment. All patients underwent a symptom limited cardiopulmonary exercise testing on a cycle-ergometer, in order to access CHF severity. A multivariate linear regression analysis using simultaneous entry of predictors was performed to examine which of the CHF variables and of the personality variables were correlated independently to QoL scores in the two summary scales of the KCCQ, namely the Overall Summary Scale and the Clinical Summary Scale.

RESULTS: The Neuroticism personality trait score had a significant inverse correlation with the Clinical Summary Score and Overall Summary Score of the KCCQ ($r = -0.621$, $P < 0.05$ and $r = -0.543$, $P < 0.001$, respectively). KCCQ summary scales did not show significant correlations with the personality traits of Extraversion, Openness, Conscientiousness and Agreeableness. Multivariate linear regression analysis using simultaneous entry of predictors was also conducted to determine the best linear combination of statistically significant univariate predictors such as Neuroticism, VE/VCO₂ slope and VO₂ peak, for predicting KCCQ Clinical Summary Score. The results show Neuroticism ($\beta = -0.37$, $P < 0.05$), VE/VCO₂ slope ($\beta = -0.31$, $P < 0.05$) and VO₂ peak ($\beta = 0.37$, $P < 0.05$) to be independent predictors of QoL. In multivariate regression analysis Neuroticism ($b = -0.37$, $P < 0.05$), the slope of ventilatory equivalent for carbon dioxide output during exercise, (VE/VCO₂ slope) ($b = -0.31$, $P < 0.05$) and peak oxygen uptake (VO₂ peak), ($b = 0.37$, $P < 0.05$) were independent predictors of QoL (adjusted R² = 0.64; F = 18.89, $P < 0.001$).

CONCLUSION: Neuroticism is independently associated with QoL in CHF. QoL in CHF is not only determined by disease severity but also by the Neuroticism personality trait.

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Key words: Chronic heart failure; Five-Factor Personality Inventory; Kansas City Cardiomyopathy Questionnaire; Quality of Life

Core tip: Of the patients with chronic heart failure (CHF), those who are experiencing low Quality of Life (QoL) show higher morbidity, hospitalization rates and mortality. There is a link between low QoL, low adherence to pharmaceutical and non-pharmaceutical treatment as well as exercise training rehabilitation, and high anxiety and depression levels. The personality of the patient has been also found to play a role in affecting QoL and therefore prognosis. Taking into account that the personality trait of Neuroticism, has been found to affect QoL in chronically ill individuals, this study explores its possible role in predicting QoL in CHF population in relation to disease severity.

Samartzis L, Dimopoulos S, Manetos C, Agapitou V, Tasoulis A, Tseliou E, Pozios I, Kaldara E, Terrovitis J, Nanas S. Neuroticism personality trait is associated with Quality of life in patients with Chronic Heart Failure. *World J Cardiol* 2014; 6(10): 1113-1121 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i10/1113.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i10.1113>

INTRODUCTION

The quantification of patients' Quality of life (QoL) is becoming a useful endpoint in the study of chronic heart failure (CHF), not only as a marker of health care quality, but also as a tool for monitoring patients over time^[1,2]. The evaluation of patients' QoL as well as of factors that are pertinent to it are considered as important for the prognosis in CHF, as QoL has been shown to identify patients with more severe syndrome^[3-6] and at greater risk for hospitalization or death^[7-9]. QoL is closely related to exercise intolerance, and exercise capacity improvement by exercise training in CHF improved QoL and reduced mortality and cardiac events^[10,11]. Furthermore, QoL in CHF has been associated with psychological factors such as depression, anxiety and personality. Depressed as well as anxious CHF patients were found to have poor QoL^[12], with depression being a strong predictor of QoL even after adjustment with disease severity^[13].

However the role of personality in the QoL of CHF has been largely overlooked, as most studies have focused on the role of depression and anxiety as the main psychological determinants. The personality trait that has been most widely studied in CHF population is type-D, which was found to be independently associated with impaired QoL in CHF^[14], anxiety and depression^[15], and increased mortality^[16]. Another well documented personality trait, Neuroticism, has been demonstrated to affect QoL in chronically ill individuals as well as to be correlated with increased levels of anxiety and depression^[17-21]. However, there have been only a few reports indicating a possible role of Neuroticism to predict QoL^[22] and prog-

nosis in CHF patients^[23,24].

Therefore, the personality trait of Neuroticism constitutes an interesting but yet not well explored factor in the relationship between personality and QoL in patients with CHF. The aim of this study is to investigate the role of Neuroticism in QoL in CHF in relation to CHF severity. We hypothesized that Neuroticism affects QoL in patients with stable CHF, independently of the disease severity.

MATERIALS AND METHODS

Study population

The study population consisted of 36 consecutive stable CHF outpatients (6 women and 30 men), with a left ventricular ejection fraction (LVEF) $\leq 45\%$ at optimal medical treatment at the time of inclusion, who were referred to our laboratory from the Heart Failure Clinic of the Medical School of the University of Athens, during the years 2008-2009, in order to perform a symptom-limited cardiopulmonary exercise test (CPET), as a part of heart failure evaluation.

Patients were excluded from the study if there was any contraindication for a CPET according to the American Thoracic Society/American College of Chest Physicians Statement on CPET^[25] and if there was a history of moderate to severe chronic obstructive pulmonary disease or cancer disease or other systemic inflammatory chronic illness. Patients enrolled in the study had no history of a known psychiatric disorder or psychiatric/psychological treatment. Patients who were on psychotropic medication or who received any form of psychotherapy treatment were excluded of the study. None of the patients had a history of psychiatric hospitalization in the past. Baseline demographic data and clinical characteristics of all patients are presented in Table 1. Informed consent was obtained from all patients, as approved by the Human Study Committee of our Institution.

Design of the study

All patients underwent an incremental symptom-limited CPET, and the day of exercise testing they were administered a questionnaire consisted of a self-rating psychometric tests battery to complete in their home. Data collection was obtained from patients' questionnaire responses and analyzed by an expert clinician to psychiatric disorders.

Cardiopulmonary exercise testing

All patients performed a symptom-limited CPET on an electro-magnetically braked cycle ergometer (Ergoline 800; Sensor Medics, Anaheim, California, United States). The work rate increment was estimated by using Hansen *et al*^[26]'s equation in order to attain test duration of 8-12 min. Measurements were recorded for 2 min at rest, for 3 min of unloaded pedaling before exercise, during exercise and for recovering period. Oxygen saturation was measured continuously by pulse oximetry, heart rate and

Table 1 Demographic data and clinical characteristics in all chronic heart failure patients (*n* = 36)

Age, yr	54 ± 12
Gender, (M/F)	30/6
BMI, kg/m ²	28.3 ± 4.9
NYHA class I / II / III	(10/20/6)
LVEF	33% ± 10%
CHF Etiology	
Non-ischemic	18 (50%)
Ischemic	18 (50%)
Medical treatment	
ACE inhibitors	88%
β-blockers	85%
Diuretics	85%
Spironolactone	52%
Amiodarone	35%
Digitalis	11%
Nitrates	20%
Antiplatelets	44%
Anticoagulants	23%

Continuous variables values are presented as means ± SD. BMI: Body mass index; NYHA: New York Heart Association; LVEF: Left ventricle ejection fraction; ACE: Angiotensin-converting enzyme; CHF: Chronic heart failure.

rhythm were monitored by a MAX1, 12-lead ECG System (Marquette), arterial pressure was measured every 2 min with a mercury sphygmomanometer. Oxygen uptake (VO₂), carbon dioxide output (VCO₂) and ventilation (VE) were measured breath-by-breath. All patients were verbally encouraged to exercise to exhaustion, as defined by intolerable leg fatigue or dyspnea.

Cardiopulmonary measurements

The gas exchange measurements served to calculate VO₂ at peak exercise (VO₂ peak, mL/kg per minute), anaerobic threshold (AT, mL/kg per minute), and VE/VCO₂ slope between exercise onset and AT. The peak values for VO₂, VCO₂, and VE were calculated as the average of measurements made during the 20-s period before exercise was terminated. AT was determined using the V-slope technique^[27], and the result was confirmed graphically from a plot of ventilatory equivalent for oxygen (VE/VO₂) and carbon dioxide (VE/VCO₂) against time. The ventilatory response to exercise was calculated as the slope by linear regression of VE *vs* VCO₂ from the beginning of exercise to AT, where the relationship is linear, calculated as in previous study^[28].

Personality measurements

Personality traits were assessed with the widely used NEO-Five Factor Inventory (NEO-FFI, Greek version), which is a 60-item self-report questionnaire based on the five factor model of personality. The NEO-FFI^[29] is a shortened version of the NEO-PI-R (Costa and McCrae 1992). Patients rate each item on a five-point Likert-like scale. Each item-score ranges on a scale from strongly disagree (0) to strongly agree (4). The instrument is designed to measure each of the well-established five

factors of personality: Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness. The Five Factor Model of personality^[29,30] is a personality concept that includes behavioral, emotional as well as cognitive personality patterns.

Neuroticism reflects distress-proneness, negative emotions and chronic emotional maladjustment, stress reactivity and instability. Extraversion refers to positive mood, sociability, need for stimulation, vigor, quantity and intensity of preferred interpersonal interactions as well as activity level and capacity for enjoyment. Openness refers to openness to experience as well as active seeking and appreciation of experiences for their own sake, and also involves entailing interest in novel ideas, aesthetic and intellectual sensibility. Agreeableness is an interpersonal factor reflecting altruism, trust, amicability, and also refers to the kind of interactions a person prefers along a continuum from compassion to antagonism. Conscientiousness involves organization, persistence, control, reliability, diligence, and also assesses the degree of achievement-orientated behavior and motivation in goal-directed behavior.

Quality of life measurements

As a measurement of QoL in CHF patients the Greek version of the Kansas City Cardiomyopathy Questionnaire was used. The KCCQ is a 23-item, disease specific questionnaire that quantifies the domains of health status that are symptoms (frequency, severity, change over time), physical limitations, a heart failure specific assessment of their quality of life and self-efficacy domain that is a measure of patients' knowledge of how to best manage their disease. The psychometric properties of the KCCQ have been well documented^[3].

The use of the KCCQ in outpatient clinical practice can both quantify patients' health status and provide insight into their prognosis^[8]. QoL identifies CHF patients at risk for hospitalization or death, as a low KCCQ score is an independent predictor of poor prognosis in patients with CHF^[7]. Studies have been shown that cross-sectional variations^[7,8] as well as changes in serial health status assessments^[9] in KCCQ scores are prognostic indicators of subsequent mortality as well as hospitalizations due to CHF.

Statistical analysis

All continuous variables are expressed as mean ± SD. A *P* value of 0.05 or lower was considered as statistically significant. All variables were tested for normal distribution. Pearson's coefficient was used to assess correlations between the study variables. A multivariate linear regression analysis using simultaneous entry of predictors was performed to examine which variables were correlated independently to QoL scores in the two summary scales of the KCCQ that are the overall summary scale (OSS) and the clinical summary scale (CSS). The Statistical Package for the Social Sciences (SPSS Statistics 17.0) software was used to analyze the data.

Table 2 Psychometric and cardiopulmonary exercise testing measurements in all chronic heart failure patients (*n* = 36)

KPLS	85 ± 14
KSSS	64 ± 21
KSFS	86 ± 16
KSBS	80 ± 19
KTSS	82 ± 17
KSES	67 ± 27
KQOLS	57 ± 24
KSLS	75 ± 26
KOSS	75 ± 17
KCSS	85 ± 14
Neuroticism	16 ± 5
Extraversion	29 ± 8
Openness	25 ± 5
Agreeableness	26 ± 6
Conscientiousness	30 ± 5
VO ₂ peak, mL/kg per minute	15.9 ± 4.4
WRp, Watt	99 ± 41
VE/VCO ₂ slope	35 ± 7
AT, mL/kg per minute	9.7 ± 2.4

KCCQ: Kansas City Cardiomyopathy Questionnaire; KPLS: Kansas Physical Limitation Score; KSSS: Kansas Symptom Stability Score; KSFS: Kansas Symptom Frequency Score; KSBS: Kansas Symptom Burden Score; KTSS: Kansas Total Symptom Score; KSES: Kansas Self-Efficacy Score; KQOLS: Kansas Quality of Life Score; KSLS: Kansas Social Limitation Score; KOSS: Kansas Overall Summary Score; KCSS: Kansas Clinical Summary Score; VO₂ peak: Peak oxygen uptake; WRp: Peak work rate; VE/VCO₂ slope: The slope of ventilatory equivalent for carbon dioxide output during exercise; AT: Anaerobic threshold.

RESULTS

Psychometric and cardiopulmonary exercise testing measurements are presented in Table 2.

Correlation analysis

The correlations between KCCQ QoL measurements and NEO-FFI personality parameters, by means of Pearson's coefficients showed a strong relationship between QoL and Neuroticism (Table 3 and Figure 1). KCCQ summary scales did not show significant correlations with the personality traits of Extraversion, Openness, Conscientiousness and Agreeableness (Table 3). QoL as measured by the OSS and CSS subscales of the KCCQ, had also a significant correlation with CHF severity (Figures 2 and 3) as expressed by VE/VCO₂ slope ($r = -0.59$, $P < 0.001$ and $r = -0.55$, $P < 0.001$, respectively) and VO₂ peak ($r = 0.56$, $P < 0.001$ and $r = 0.65$, $P < 0.001$, respectively).

Model of predictors of Quality of life

A univariate linear regression was performed to examine which variables were significantly correlated to QoL scales, including age, Body Mass Index, Personality traits, VE/VCO₂ slope and VO₂ peak.

Multivariate linear regression analysis using simultaneous entry of predictors was conducted to determine the best linear combination of statistically significant univariate predictors such as Neuroticism, VE/VCO₂ slope and VO₂ peak, for predicting KCCQ Overall Summary Score.

Table 3 Pearson's Simple Correlations between Kansas City Cardiomyopathy Questionnaire Quality of Life and NEO Five-Factor Inventory personality parameters

KPLS	-0.53 ^b	-0.01	0.31	-0.15	-0.03
KSSS	-0.30	-0.02	-0.06	-0.17	-0.15
KSFS	-0.54 ^b	-0.31	-0.09	-0.41	-0.24
KSBS	-0.57 ^b	-0.27	-0.15	-0.27	-0.37
KTSS	-0.61 ^b	-0.29	-0.09	-0.35	-0.32
KSES	0.00	-0.14	-0.24	-0.28	-0.42
KQOLS	-0.38 ^a	-0.13	-0.02	-0.26	-0.31
KSLS	-0.39 ^a	-0.12	0.13	0.02	0.10
KOSS	-0.54 ^b	0.05	0.08	-0.19	-0.07
KCSS	-0.62 ^b	-0.08	0.10	-0.28	-0.10
	Neuroticism	Extraversion	Openness	Agreeableness	Conscientiousness

The statistical significance was set at P value < 0.05 . ^a $P < 0.05$; ^b $P < 0.01$. KCCQ: Kansas City Cardiomyopathy Questionnaire; KPLS: Kansas Physical Limitation Score; KSSS: Kansas Symptom Stability Score; KSFS: Kansas Symptom Frequency Score; KSBS: Kansas Symptom Burden Score; KTSS: Kansas Total Symptom Score; KSES: Kansas Self-Efficacy Score; KQOLS: Kansas Quality of Life Score; KSLS: Kansas Social Limitation Score; KOSS: Kansas Overall Summary Score; KCSS: Kansas Clinical Summary Score; NEO-FFI: NEO Five-Factor Inventory.

The results show that Neuroticism ($\beta = 0.32$, $P < 0.05$), VE/VCO₂ slope ($\beta = 0.41$, $P < 0.05$) are independent predictors of QoL (adjusted $R^2 = 0.51$, $F = 13.33$, $P < 0.001$), with VO₂ peak showing a trend (Table 4).

Multivariate linear regression analysis using simultaneous entry of predictors was also conducted to determine the best linear combination of statistically significant univariate predictors such as Neuroticism, VE/VCO₂ slope and VO₂ peak, for predicting KCCQ Clinical Summary Score. The results show Neuroticism ($\beta = -0.37$, $P < 0.05$), VE/VCO₂ slope ($\beta = -0.31$, $P < 0.05$) and VO₂ peak ($\beta = 0.37$, $P < 0.05$) to be independent predictors of QoL (Table 4). Even after accounting for CHF etiology, no changes emerged concerning the predictors in the two models described above.

DISCUSSION

This study provides confirming evidence for the hypothesis that personality factors affect QoL in CHF. More specifically, in our study the personality trait of Neuroticism is associated with QoL independently of the CHF severity. To our knowledge this study is the first to show that the personality trait of Neuroticism, estimated by the NEO-FFI, affects QoL, in CHF patients after adjustment for disease severity evaluated by a symptom-limited cardiopulmonary exercise test.

Previous researchers have reported that Neuroticism, estimated by the Eysenk Personality Inventory, can predict the mental health component of the generic QoL, after adjustment for disease severity (assessed by the 6-min walk distance)^[22]. A positive correlation between Neuroticism and depression was also found by the same research group in CHF patients^[24]. In a prospective cohort study, Murberg *et al*^[23] using the Eysenk Personality Questionnaire have shown that Neuroticism, predicts mortality

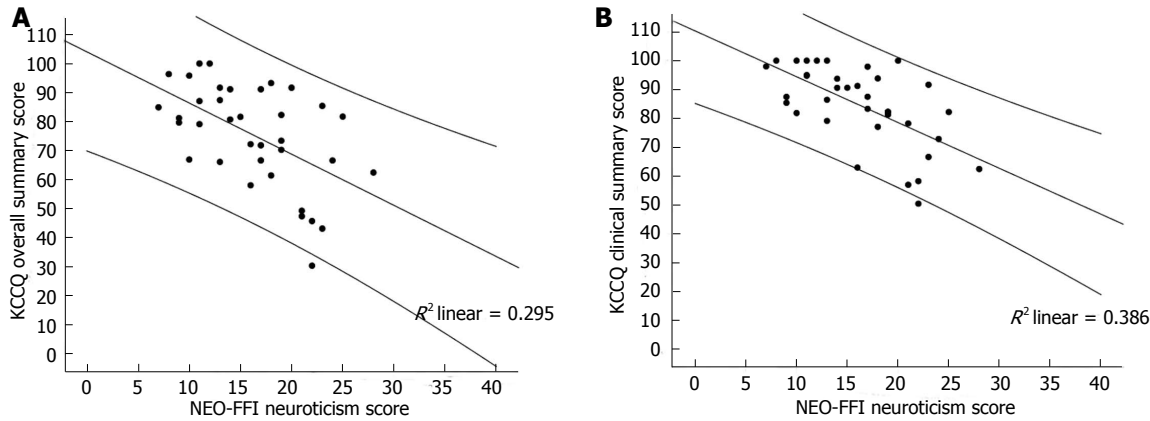


Figure 1 Scattergrams of correlation of Quality of Life subscales (Kansas City Cardiomyopathy Questionnaire Overall Summary Score, Kansas City Cardiomyopathy Questionnaire Clinical Summary Score) with NEO- Five-Factor Inventory Neuroticism personality trait (A and B, respectively).

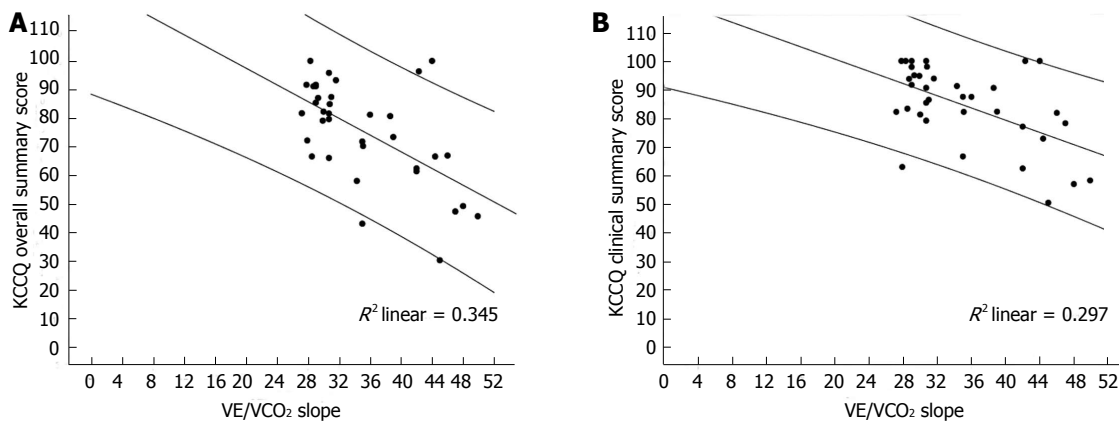


Figure 2 Scattergrams of correlations of Quality of Life (Kansas City Cardiomyopathy Questionnaire Overall Summary Score, Kansas City Cardiomyopathy Questionnaire Clinical Summary Score) with VE/VCO₂ slope (A and B, respectively).

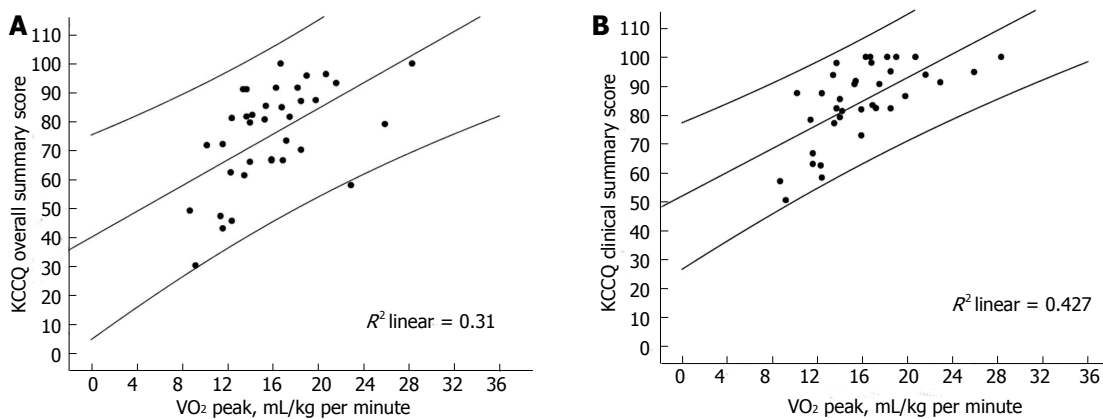


Figure 3 Scattergrams of correlation of Quality of Life (Kansas City Cardiomyopathy Questionnaire Overall Summary Score, Kansas City Cardiomyopathy Questionnaire Clinical Summary Score) with VO₂ peak (A and B, respectively).

in CHF independently of the disease severity (assessed by the pro-ANP biochemical prognostic marker). In our study, the Neuroticism trait emerged as an independent predictor of QoL in CHF patients, even after adjustment for the most robust prognostic indicators of mortality, namely cardiopulmonary exercise, stress test parameters, confirming previous reports. Furthermore, CHF patients enrolled at the present study were receiving modern op-

timal medical treatment including beta-blockers, ACE-inhibitors, aldosterone antagonists.

Recently, other studies have examined the relationship between personality and QoL and demonstrated that type D personality is independently associated with impaired health status in CHF^[14,31]. Previous data have also supported the finding that the relationship between personality type-D and CHF is not confounded by dis-

Table 4 Multiple linear regression analyses of predictors of Quality of Life

Variables	KCCQ summary scales							
	KCCQ Overall Summary Score adjusted $R^2 = 0.51$; $F = 13.33$, $P < 0.001$				KCCQ Clinical Summary Score adjusted $R^2 = 0.64$; $F = 18.89$, $P < 0.001$			
	B	SE	beta	Sig.	B	SE	beta	Sig.
Neuroticism	-1.02	0.43	-0.32	$P < 0.05$	-0.95	0.31	-0.37	$P < 0.05$
VE/VCO ₂ slope	-1.01	0.32	-0.41	$P < 0.05$	-0.6	0.23	-0.31	$P < 0.05$
VO ₂ peak	1.04	0.55	0.26	NS	1.16	0.39	0.37	$P < 0.05$

KCCQ: Kansas City Cardiomyopathy Questionnaire; VE/VCO₂ slope: The slope of ventilatory equivalent for carbon dioxide output during exercise; VO₂ peak: Peak oxygen uptake.

ease severity, assessed by BNP measurements^[32]. In the present study we have shown that the personality trait of Neuroticism, affects independently QoL in CHF patients. The relationship between type-D personality and Neuroticism has been previously studied and a positive relationship was found between them in both subscales of the type-D evaluation tool DS14, namely the Negative Affectivity and Social Inhibition^[33]. Other psychological factors such as depression and anxiety have also been associated with decreased QoL in CHF patients^[12].

Our data have shown that CHF patients who score high on the Neuroticism scale have low QoL independently of the severity of the CHF as expressed by VE/VCO₂ slope and VO₂ peak, strong predictors of mortality^[28]. Because of the cross-sectional design of the study, the results are by definition bidirectional. Nevertheless it could plausibly be assumed that the direction of the relationship is rather from Neuroticism to impaired QoL, as personality traits are usually formed before the age of 30 and tend to remain stable through the rest of adulthood^[34-37]. As supported by epidemiological data, CHF usually occurs mainly after the age of 30, increasing incidence with aging^[38,39]. Thus it can be inferred that the development of Neuroticism personality trait precedes the development and progress of CHF.

Chronic stress pattern and autonomic dysregulation might explain the relationship between Neuroticism and QoL found in our data. The personality trait of Neuroticism is correlated to chronic anxiety^[40]. Neuroticism leads to a dysfunctional pattern of stress management as well as a pattern of frequent experience of negative feelings in everyday life, and that means vulnerability to common psychological stressors. This chronic inadequate stress management predisposes to autonomic nervous dysfunction^[41] and cardiovascular dysregulation^[42,43]. The relationship between chronic stress and cardiovascular dysfunction is also well known in animals^[44]. A recent study has shown that type-D personality is correlated to impaired heart rate recovery^[45], an index of parasympathetic abnormality. This index is related to psychological distress and QoL and is a strong independent predictor of mortality^[46] and poor QoL^[47] in CHF patients. It could be assumed that this autonomic nervous system dysregulation is a possible mechanism that might mediate the relationship between Neuroticism and decreased health status in CHF patients. Future studies are needed to evaluate the

possible association of Neuroticism with autonomic nervous system impairment in CHF patients.

Our study supports the hypothesis that a more holistic approach is preferable, when evaluating a patient with chronic heart failure, a finding of significant clinical importance. A low KCCQ score is an independent predictor of poor prognosis in outpatients with CHF^[7-9] and according to the findings of present study, this is not only related to disease severity but also predicted by a high NEO-FFI Neuroticism score. Patients scoring high in the Neuroticism personality trait have lower QoL independently of CHF severity, therefore, Neuroticism may constitute a prognostic factor for these patients. The knowledge of the severity of the Neuroticism trait could also affect the treatment options, as it can help with the identification of patients that probably need additional psychological or psychiatric care to cope with their heart disease.

Certain limitations of the study should be taken into account during result interpretation. This study is cross-sectional, therefore not designed to prove causality. Intervention studies targeting Neuroticism with prospective design are needed for clarifying the direction of the relationships between Neuroticism, CHF severity and QoL, in terms of their predictive value for clinical outcome. No structured clinical interview was used as a screening tool for psychopathology in this non-psychiatric setting sample of ambulatory patients of the outpatient Heart Failure Clinic. Due to the small number of female patients in our sample, it was not possible to perform gender-specific analysis and/or between-gender comparisons. Duration of disease variables was not included in the analysis, but disease severity estimation was based only on current measurements *via* the CPET and the psychometric evaluation. Although duration of diagnosis it is not theoretically possible to affect personality traits of the patients, it might affect QoL. Due to the small sample size it was not possible to control for anxiety and depression levels. Larger sample size and sophisticated statistical methods could help to further highlight the association between Neuroticism, CHF severity and QoL. A prospective study of exercise rehabilitation program effects could lead to better exploration of the role of Neuroticism to QoL and disease severity. The role of Neuroticism in other chronic illnesses beyond CHF should be also investigated in future studies.

In conclusion, Neuroticism personality trait predicts independently QoL in CHF patients after adjustment for CHF severity. Notwithstanding the limitations of a small study, we propose that a more flexible approach to CHF diagnosis, which includes personality dimensions along with a description of CHF symptoms, may result in a more inclusive and useful diagnostic scheme for treating people with chronic heart failure. Taking into account the relationship between Neuroticism and QoL, personality factors could probably help to explain at least partially some of the mortality risk in CHF patients previously predicted by poor QoL. Psychiatric interventions might possibly be incorporated into the treatment of these patients to improve QoL and possibly prognosis.

COMMENTS

Background

Measuring and exploring factors that affect Quality of life (QoL) is important for chronic heart failure (CHF) patients in order to monitor their treatment course and assessing prognosis, as well as for evaluating treatment interventions' and health services effectiveness. Patients that are experiencing low QoL present higher morbidity, hospitalization rate and mortality. QoL in CHF is related to exercise capacity and adherence to rehabilitation programs and low QoL is related to exercise training intolerance. Of the psychological factors that affect QoL, anxiety and depression levels have been shown to play an important role, but the role of personality traits of the CHF patient hasn't been well understood yet. Taking into account that the personality trait of Neuroticism, has been demonstrated to affect QoL in chronically ill individuals, there is a need for exploration of its possible role as a predictor of QoL in patients with CHF.

Research frontiers

Of the available generic and disease-specific tools for describing dimensions of QoL in CHF, the Kansas City Cardiomyopathy Questionnaire is a self-reported, disease specific questionnaire that is considered reliable and valid for this population. Cardiopulmonary exercise testing has emerged over the years as a very useful modality for assessing variables related to exercise capacity and with CHF severity in this population.

Innovations and breakthroughs

Previous researchers have reported that Neuroticism, estimated by the Eysenk Personality Inventory (EPI), can predict the mental health component of the generic QoL, after adjustment for disease severity assessed by the 6-min walk distance. Also a prospective cohort study using the Eysenk Personality Questionnaire (EPQ), an expanded version of EPI, has shown that Neuroticism predicts mortality in CHF independently of the disease severity assessed by the pro-ANP biochemical prognostic marker. These study provides additional evidence for the hypothesis that personality factors affect QoL in CHF. More specifically, these found that the personality trait of Neuroticism is associated with QoL independently of the CHF severity. To these knowledge this study is the first to show that the personality trait of Neuroticism, estimated by the Five-Factor Personality Inventory, affects QoL, in CHF patients after adjustment for disease severity evaluated by a symptom-limited cardiopulmonary exercise test, the "gold" standard for the assessment of exercise capacity in these patients.

Applications

The results of this study showed that not only disease severity but also the personality characteristic of Neuroticism can affect patients' QoL, therefore worsening prognosis of CHF. The personality of CHF patient has to be taken into account during CHF treatment and rehabilitation programs, and a tailor-made individualized psychosocial intervention by a mental health professional might help improving patients QoL and consequently CHF prognosis.

Terminology

CHF population in this manuscript refers to patients with Chronic Heart Failure, that are currently been stabilized in optimal medical treatment and their left ventricle ejection fraction remains less than 45%. CPET refers to CardioPulmonary Exercise Testing that is used for evaluation of the patient in terms of heart function and exercise capacity. This evaluation is also important for exercise training rehabilitation of the CHF patient.

Peer review

This is a very interesting and novel observational study of the effect of neuroticism on quality of life of a Greek cohort of chronic heart failure patients. The paper reads well overall and reports some novel findings.

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Aorto-right atrial fistula: Late complication of tricuspid valve infective endocarditis

Pedro A Villablanca, Shashvat Sukhal, Oscar Maitas, Afiachuukwu Onuegbu, Juan M Muñoz-Peña, Ajay Joseph, Carlos Requena, Divyanshu Mohananey

Pedro A Villablanca, Shashvat Sukhal, Oscar Maitas, Afiachuukwu Onuegbu, Juan M Muñoz-Peña, Ajay Joseph, Carlos Requena, Divyanshu Mohananey, Department of Internal Medicine, John H. Stroger, Jr. Hospital of Cook County, Chicago, IL 60612, United States

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Correspondence to: Pedro A Villablanca, MD, Department of Internal Medicine, John H. Stroger Jr. Hospital of Cook County, 1900 West Polk Street, 15th Floor, Chicago, IL 60612, United States. pedrovillablanca@hotmail.com

Telephone: +1-773-4566609 Fax: +1-312-8649725

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ated to prosthetic aortic valve infective endocarditis. The median duration of symptoms to echocardiographic detection of fistulization is about one month. We present a case of aorto-atrial fistula at late presentation, 30 years after tricuspid valve infective endocarditis. This article describes the epidemiology, clinical manifestations, pathophysiology, diagnostic modalities, treatment and outcomes of aorto-cardiac fistulas.

Villablanca PA, Sukhal S, Maitas O, Onuegbu A, Muñoz-Peña JM, Joseph A, Requena C, Mohananey D. Aorto-right atrial fistula: Late complication of tricuspid valve infective endocarditis. *World J Cardiol* 2014; 6(10): 1122-1126 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i10/1122.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i10.1122>

Abstract

Abnormal connections between the ascending aorta and the cardiac chambers are rare, especially in the context of right-sided infective endocarditis (IE). Trans-thoracic echocardiography (TTE) with color-flow Doppler, transesophageal echocardiography (TEE), or both may be required for diagnosis. We present the case of a woman admitted with right-sided heart failure (HF) symptoms. She had a previous history of tricuspid valve IE 30 years ago. TTE and TEE revealed an aorto-right atrium fistula located just under the non-coronary cusp into the right atrium at the level of the previously affected tricuspid valve. The Patient refused surgery and was discharged home on HF medications. She has been stable for the last 3 years. The peculiarity of this case is the late symptomatic presentation of the aorto-atrial fistula and the unusual association to tricuspid valve IE.

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Key words: Aorto-cardiac fistula; Infective endocarditis; Tricuspid valve

Core tip: Aorto-cardiac fistulas are rare, usually associ-

INTRODUCTION

Aorto-cardiac fistulas (ACF) are a rare complication of infective endocarditis (IE); it is usually a complication of prosthetic aortic valve IE. We report a case of a patient who was found to have an Aorto-right atrial fistula 30 years after his tricuspid valve IE was treated. No similar late complication of tricuspid valve IE has been reported.

CASE REPORT

A 51-year-old woman presented to the emergency department (ED) with worsening decreased exercise tolerance over the past 2 mo. Her past medical history was significant for a previous culture-negative tricuspid valve IE in 1980 that was treated medically with antibiotics, permanent atrial fibrillation, asthma and hypothyroidism. Home medications included aspirin, furosemide, metoprolol and albuterol.

The patient stated that in the last 2 mo she developed worsening shortness of breath, lower extremity edema, chest tightness, palpitations, weakness-fatigue and

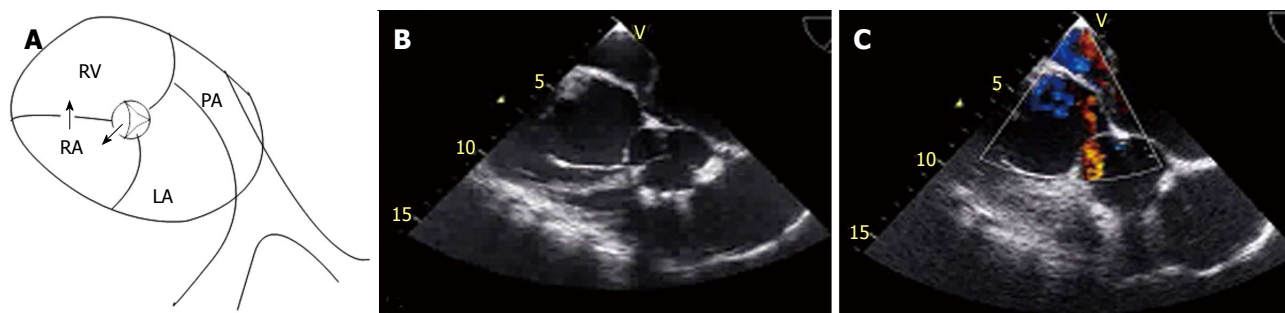


Figure 1 Schematic of abnormal fistula flow and transesophageal doppler imaging. A: Schematic diagram indicating the flow of blood during diastole, as showed by the transesophageal echocardiogram with Doppler; B: Transesophageal 4 chamber view at the level of the aortic valve, with chamber anatomy corresponding to schematic in A; C: Diastolic blood into the RA from the non-coronary cusp of the aorta. RA: Right atrium; RV: Right ventricle; LA: Left atrium; PA: Pulmonary artery.

abdominal discomfort. She denied any inciting events. Within the last two weeks, her New York Heart Association (NYHA) functional class deteriorated from NYHA class I to NYHA class III, manifested by shortness of breath on minimal exertion, relieved only by rest. The patient denied orthopnea, paroxysmal nocturnal dyspnea, hemoptysis, chest pain, fevers, chills and weight loss.

On physical examination, her heart rate was 134 beats/min, blood pressure 115/62 mmHg and a temperature of 36 °C. Cardiac examination revealed a markedly elevated jugular venous pressure of 12 cm, grade 4/6 low frequency pansystolic murmur in the lower left sternal border, irregularly irregular rhythm, hepatojugular reflux and lower extremity edema up to the knees. The rest of the physical examination was normal.

Laboratory work up included BMP, CBC, ESR, CRP, which were unremarkable, except for microcytic anemia of 8.2 g/dL with normal ferritin and a BNP of 1046 pg/dL. The EKG demonstrated atrial fibrillation, right axis deviation and an incomplete right bundle branch block. The chest X-ray was significant for moderate cardiomegaly with right atrial and ventricular enlargement. She was started on diuretics and beta-blockers for heart failure (HF) exacerbation secondary to atrial fibrillation with rapid ventricular response and then transferred to the general medicine floor.

On the next day of admission, a transthoracic echocardiography (TTE) was performed, showing normal left ventricular size and systolic function, severe dilation of the right ventricle with mark hypocontractility, severe tricuspid regurgitation with the anterior leaflet calcified with flail segments from previous IE. There was a diastolic flow into the right atrium from the aorta (non coronary cusp). There was no evidence of aortic insufficiency, out-flow tract gradient, or ventricular septal defect. Given our suspicion for aorto-atrial fistula, a transesophageal echocardiogram (TEE) was done, showing a small fistula from just under the non-coronary cusp into the right atrium at the level of the previously affected tricuspid valve (Figure 1). The patient underwent cardiac catheterization, which showed normal coronary arteries and confirmed the echocardiographic findings of an Aorto-right atrial fistula and tricuspid regurgitation.

Cardiac surgery service was consulted for tricuspid valve replacement and repair of the fistula. Patient refused surgery due to religious issues and was discharged home on diuretics, beta-blockers, angiotensin receptor 2 blockers and spironolactone. At 3-year follow up, patient has been stable, with no further exacerbation. Surgery has been offered repeatedly, we explained the risks and benefits of performing the surgery and the good results that can be accomplished with an acceptable morbidity and mortality, yet she only wants to continue with medical management.

DISCUSSION

IE has been associated with a myriad of complications such as HF and stroke^[1]. The frequency and type of complications due to IE have changed with advances in diagnosis and therapy. Uncontrolled rare extra-valvular cardiac complications of IE, such as fistulous intra-cardiac connections, which were previously common complications of IE, are infrequent in the antibiotic era. Reported for the first time in 1924 as an incidental finding on autopsy^[2], the incidence of ACF has been described in less than 2.2% of cases of native valve IE^[3] and 3.3% of prosthetic valve IE^[4] in retrospective studies. *Staphylococcus aureus* has been documented as the most common etiology reported on both autopsies and retrospective studies^[5-5] with *Streptococcus spp.*, *Enterococcus spp.*, and other bacterial and fungal infections as other documented etiologies^[6].

ACF has been documented in a variety of clinical scenarios, most frequently occurring in cases of aortic valve IE, and is more common in prosthetic than native aortic valve. ACF is present in less than 1% of right-sided IE cases, and is usually associated with concomitant native aortic IE^[7,8]. There are isolated cases in the English literature that report ACF secondary to native tricuspid valve^[9]. It has been described also with blunt trauma^[10], stab wound of the chest^[11], ruptured aneurysms of the sinus of valsalva (SV)^[12], aortic dissection^[13], congenital disorder^[14], cardiac valve surgery^[15], percutaneous cardiac valve implantation^[16], heart transplantation^[17], and autoimmune vasculitis^[18].

The proposed theory to explain the fistulization mechanism between the aorta and the cardiac chamber is through the bacterial invasion and spread of the affected valve into the adjacent tissues and structures, resulting in the formation of a periannular abscess and erosion of the SV. The aortic abscesses involving the SV may rupture internally with erosion of the sinus and subsequent development of aorto-cavitary or aorto-pericardial fistulas^[3,5,7,19,20]. Perivalvular abscesses have been reported as the cause of 6%-9% of fistula cases^[21,22]. Due to its relative avascularity and infected regurgitation of jet striking subvalvular structures^[23], the intervalvular fibrosa is more susceptible to infection^[24]. The ACF creates a left to right shunt from any of the three aortic valve sinuses to any of the four cardiac chambers with no preponderance from any specific aortic sinus to a specific cavity, resulting in further hemodynamic deterioration^[5,7]. These pathologic communications are highly morbid and lead to hemodynamic instability secondary to the shunt effect^[19].

Diagnosing ACF can be challenging, and the clinical presentation will depend on the size of the shunt. Patients with a small ACF may be completely asymptomatic with an associated murmur only^[25,26], but the clinical presentation may range from refractory HF^[20] to a chest pain syndrome due to acute coronary syndrome and aortic dissection^[17,27]. Cardiac auscultation may cause a continuous murmur^[28], a thrill^[29] or both^[25], and can be the key to further pursue this diagnosis with appropriate imaging modalities. The median duration of symptoms to echocardiographic detection of fistulization is about 25 d as reported in a retrospective multi-center study^[7]. There are isolated cases reported years after prosthetic valve implantation^[15]. A high index of suspicion is required, especially in the background of recent surgery or previous IE.

Although aortography is the gold standard for diagnosis, non-invasive methods such as contrast enhanced CT, MRI, and echocardiography are currently preferred. TTE is the initial test of choice in the routine assessment of patients presenting with HF symptoms or murmurs, and is therefore usually the first image modality that allows us to confirm or suspect the presence of an ACF. However, TEE is superior to TTE for better delineation of function and morphology when intra-cardiac complications, such as ACF, are suspected^[30,31]. The high rate of echocardiographic diagnosis is likely due to the high-pressure differences between the aorta and the cardiac chambers, which enables observation of the highly turbulent flow that is easily detectable by color Doppler^[7]. Three-dimensional echocardiography has been reported to have the potential to delineate anatomic structures, allowing a greater understanding of the pathological process and also obtaining unconventional views of cardiac structures^[32]. It can delineate structures that are otherwise not visible in TEE and TTE, allowing cropping, full-volume data; and slicing in various planes^[33,34]. Computed tomography, magnetic resonance imaging, and aortography can allow better description, position, dimension and anatomic conditions of the ACF, and may be required as an important adjunctive tool to confirm the diagnosis

and delineate the anatomy before closure^[35-38].

Surgery, which is the primary treatment of ACF, may carry severe complications, particularly with critically unstable patients with an increased postoperative mortality after surgical correction^[4]. Factors associated with adverse outcomes include staphylococcal infection, urgent or emergency surgery, moderate to severe HF, renal failure, increased age and residual fistula^[3,7,19,20,22,39]. With the high postoperative mortality with surgical closure of ACF and with the advancement of endovascular technologies, more emphasis is now placed on percutaneous closure with devices such as an Amplatzer plug^[40,41], though it should be avoided in patients with active infection.

We report a case of a patient who was found to have an Aorto-right atrial fistula 30 years after his tricuspid valve was treated for IE. To our knowledge and after a systematic review of the English literature, no similar late complication of treated IE has been reported.

COMMENTS

Case characteristics

A 51-year-old female with a history of tricuspid valve infective endocarditis presented with shortness of breath.

Clinical diagnosis

Right side heart failure symptoms.

Differential diagnosis

Aorto-cardiac fistula vs valvular heart disease vs new infective endocarditis.

Laboratory diagnosis

Hb of 8.2 g/dL with normal ferritin and a BNP of 1046 pg/dL; inflammatory markers (erythrocyte sedimentation rate, serum C-reactive protein, blood cell count) were within normal limits.

Imaging diagnosis

Transthoracic and transesophageal echocardiography demonstrated Aorto-right atrial fistula.

Treatment

The patient was medically managed for her heart failure after she refused surgical treatment.

Related reports

Echocardiography images and explanatory figure are provided in the case report.

Experiences and lessons

A high index of suspicion for aorto-cardiac fistula is required, especially in the background of recent surgery or previous infective endocarditis.

Peer review

This is an interesting paper.

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Electrical storm in systemic sclerosis: Inside the electroanatomic substrate

Michela Casella, Corrado Carbuicchio, Eleonora Russo, Francesca Pizzamiglio, Paolo Golia, Sergio Conti, Fabrizio Costa, Antonio Dello Russo, Claudio Tondo

Michela Casella, Corrado Carbuicchio, Eleonora Russo, Francesca Pizzamiglio, Sergio Conti, Antonio Dello Russo, Claudio Tondo, Cardiac Arrhythmia Research Centre, Centro Cardiologico Monzino IRCCS, 20138 Milan, Italy
Paolo Golia, Department of Cardiovascular Disease, Morgagni-Pierantoni Hospital, 27121 Forlì, Italy

Fabrizio Costa, Biosense-Webster, 00040 Pomezia, Italy

Author contributions: Casella M, Carbuicchio C and Dello Russo A contributed to conception and design, acquisition of data, analysis and interpretation of data, drafting the article, final approval of the version to be published; Russo E, Golia P and Costa F contributed to conception and design, acquisition of data, analysis and interpretation of data; Pizzamiglio F and Conti S contributed to drafting the article; Tondo C final approval of the version to be published.

Correspondence to: Michela Casella, MD, PhD, Cardiac Arrhythmia Research Centre, Centro Cardiologico Monzino, IRC-CIS, Via Parea 4, 20138 Milan, Italy. michela.casella@ccfm.it

Telephone: +39-02-58002340 Fax: +39-02-58002398

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Abstract

We report the case of a 63-year-old woman affected by a severe form of systemic scleroderma with pulmonary involvement (interstitial fibrosis diagnosed by biopsy and moderate pulmonary hypertension) and cardiac involvement (paroxysmal atrial fibrillation, right atrial flutter treated by catheter ablation, ventricular tachyarrhythmias, previous dual chamber implantable cardioverter defibrillator implant). Because of recurrent electrical storms refractory to *iv* antiarrhythmic drugs the patient was referred to our institution to undergo catheter ablation. During electrophysiological procedure a 3D shell of cardiac anatomy was created with intracardiac echocardiography pointing out a significant right ventricular dilatation with a complex aneurysmal lesion characterized by thin walls and irregular multiple trabeculae. A substrate-guided strategy of catheter ab-

lation was accomplished leading to a complete electrical isolation of the aneurism and to the abolishment of all abnormal electrical activities. The use of advanced strategies of imaging together with electroanatomical mapping added important information to the complex arrhythmogenic substrate and improved efficacy and safety.

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Key words: Ventricular tachycardia; Electrical storm; Radiofrequency catheter ablation; Systemic sclerosis

Core tip: We report the case of a 63-year-old woman affected by a severe form of systemic scleroderma with cardiac involvement. Because of recurrent electrical storms the patient underwent catheter ablation. Intracardiac echocardiography pointed out a significant right ventricular dilatation with a complex aneurysmal lesion characterized by thin walls and irregular multiple trabeculae. A substrate-guided strategy of catheter ablation was accomplished leading to a complete electrical isolation of the aneurism. The use of advanced strategies of imaging together with electroanatomical mapping added important information to the complex arrhythmogenic substrate and improved efficacy and safety.

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INTRODUCTION

Systemic sclerosis (SS) is a rare systemic infiltrative dis-



Figure 1 Movies. A: Intracardiac echocardiography (ICE) imaging showing right ventricle (RV) aneurysmal dilatation; B: At ICE, left ventricle was of normal size but with diffuse parietal hypertrophy and a mild pericardial effusion around the mitral valve plane; C: Right ventricular (RV) angiography in right anterior oblique projection.

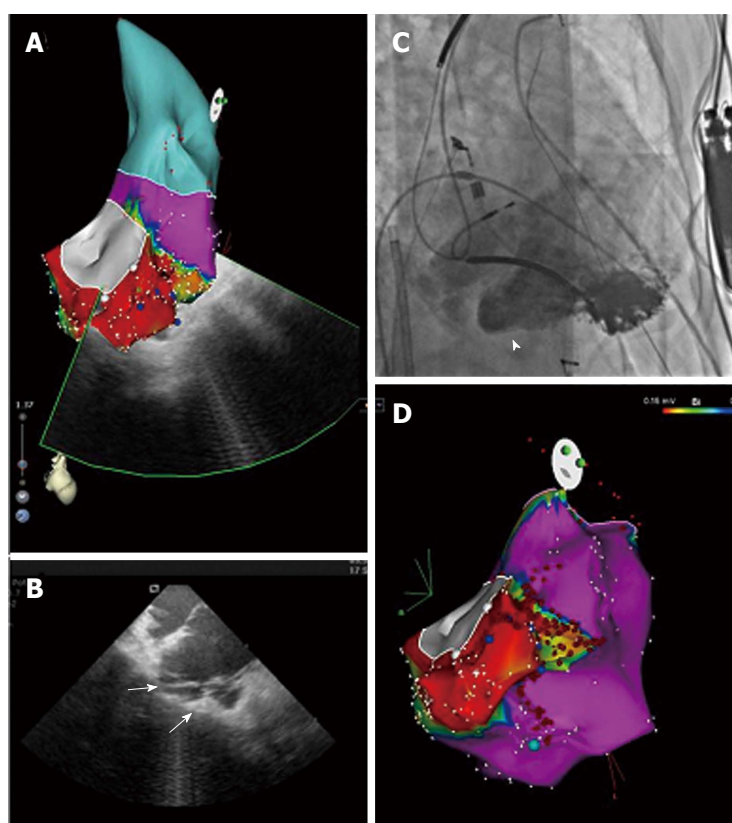


Figure 2 Right ventricle imaging. A: Bipolar voltage map of the right ventricle (RV) in right anterior oblique view. The bipolar potential voltage values were normal in the whole ventricle (purple) except for a wide area (red) around the inferior-lateral portion of tricuspid valve; B: Intracardiac echo fan intersecting the low-voltage area, showing aneurysmal dilatation (arrows) characterized by thin walls and irregular multiple trabeculae; C: RV angiography confirming the presence of the aneurysmal dilatation (head arrow); D: substrate map in right anterior oblique view showing the RF lesion points. CA was guided by substrate map and was performed all along the borders of the aneurysm and extended to the peri-aneurysmal area leading to abolishment of all abnormal electrical activities.

order characterized by a widespread damage to small blood vessels and connective tissue fibrosis resulting in multi-organ involvement. Ventricular tachyarrhythmias (VTs) are frequent clinical manifestation of SS-associated cardiovascular damage and a possible cause of sudden death. Antiarrhythmic therapy is usually limited by concomitant therapy or side effects. In many cases the use of an implantable cardioverter defibrillator (ICD) is mandatory. In this setting catheter ablation (CA) has been proposed as an alternative option but no evidence exists about the characteristics of the arrhythmogenic substrate.

CASE REPORT

A 63-year-old woman affected by a severe form of SS was referred to our institution for management of her VTs. She presented both an advanced pulmonary and cardiac involvement. Since June 2006 she has been suffering

from multiple episodes of sustained VT; pharmacological therapy by beta blockers, sotalol or amiodarone was ineffective and limited by side effects (Raynaud's phenomenon and pulmonary interstitial fibrosis) thus the patient underwent a dual chamber ICD implant; on September 2013 the patient developed recurrent electrical storms refractory to *in situ* antiarrhythmic drugs.

On the second day after admission, CA was performed. First, a 3D shell of cardiac anatomy was created on an electroanatomic mapping system integrated with intracardiac echocardiography (ICE) (Cartosound, Biosense-Webster, United States)^[1]. ICE allowed to detect a significant right ventricular (RV) dilatation with a complex lesion characterized by thin walls and irregular multiple trabeculae, expanding from the inferior to the lateral RV wall in perivalvular basal segments (Figure 1A and B) this aneurysmal dilatation was easily identified angiographically, too (Figure 1C, Figure 2).

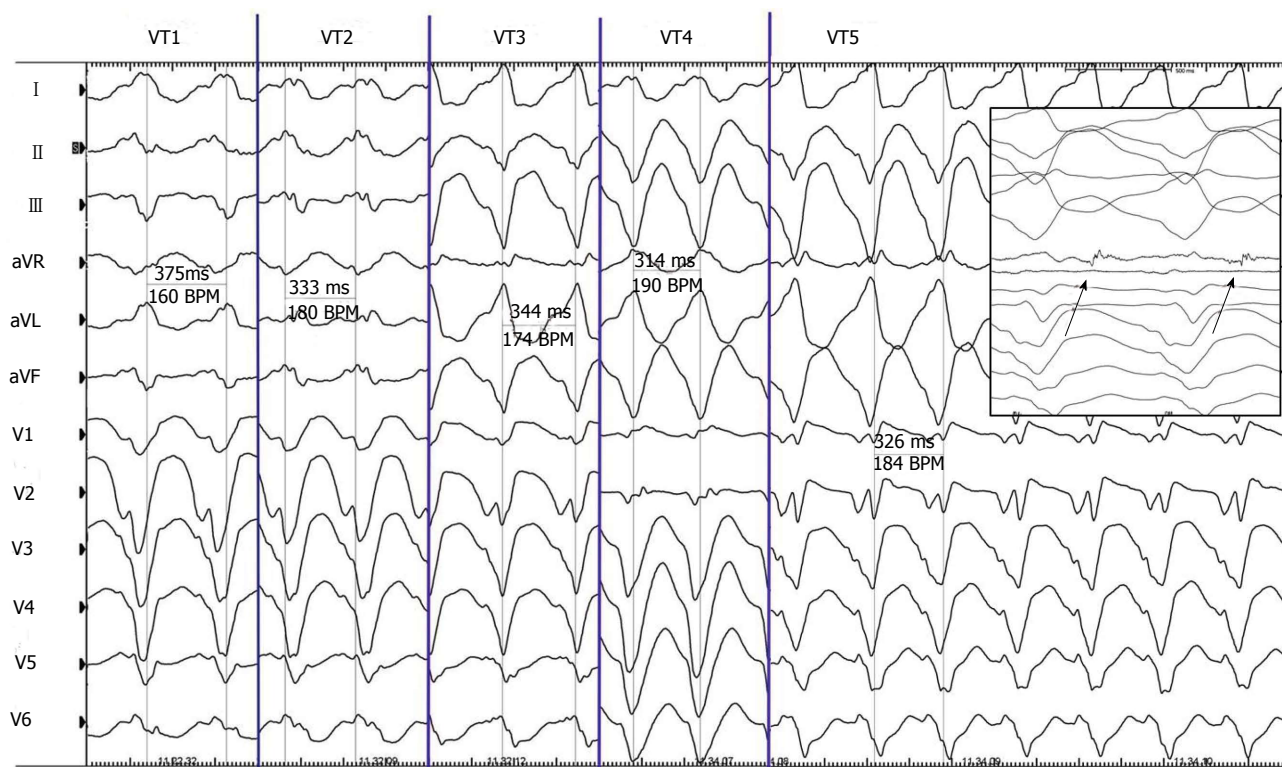


Figure 3 Cycle and QRS morphologies of all the 5 sustained ventricular tachyarrhythmias observed and mapped during the procedure. In the box it is possible to appreciate the mid-diastolic potential (arrows) recorded during ventricular tachyarrhythmias (VT) 5. BPM: Beat per minute.

A complete electroanatomical map (EAM) of the aneurysm was obtained by ICE, showing an area of dense scar surrounded by near-scar tissue with abnormal electrical activities (AEAs) all along the borders of the aneurysm. Five VT morphologies spontaneously occurred (Figure 3); only 2 of them were effectively mapped creating an activation map and identifying a mid-diastolic potential, both were terminated by radiofrequency (RF) delivery at the critical isthmus located at the border of the aneurysm. Then a substrate-guided CA was accomplished targeting all AEAs by sequential RF energy pulses (30 up to 40 Watts, SF Thermocool, Biosense-Webster, United States), to achieve the complete electrical isolation of the aneurysm. At the end of the procedure, a complete protocol of programmed electrical stimulation (drive 600 and 400 ms, up to three extrastimuli) from the RV apex was negative. No VT recurrence was observed in a 6 mo follow up period on amiodarone (1 g/wk) therapy.

DISCUSSION

Tachyarrhythmias appear as frequent clinical manifestations of SS-associated cardiovascular damage. Arrhythmias occurrence may be associated with poor outcome and represent 6% of the overall causes of death in the large European League Against Rheumatism Scleroderma Trials and up to 12% in Research (EUSTAR) database^[2].

Since different classes of anti-arrhythmics are available and SS patients may have multiple organs involved and take concomitant drugs, the choice of treatment

must be personalized to the patient. ICDs have been used effectively in selected patients to prevent sudden cardiac death. There is no specific recommendation in VT treatment in SS patients, and the use of CA has been reported anecdotally^[3,4]. We present the first case of SS patient with multiple VT morphologies undergoing successful CA guided by an integrated approach aiming at the electro-anatomical characterization of RV cardiomyopathy. Based on our experience CA should be considered as an adjunctive treatment in patients with sustained, monomorphic VTs refractory to pharmacological therapy. Our experience adds new pieces on the knowledge of the arrhythmic substrate in SS. First of all, CA approach requires an accurate imaging of the RV acquired by both angiography and real time echo (ICE, as in our case, or transoesophageal) to identify the area of interest and to reduce potential risk as RF delivery in very thin tissue^[5]. Secondary, a combined high density mapping of the whole scar and peri-scar area allows a substrate-guided abolition of all AEAs leading to a successful procedure.

Malignant VTs may be expression of an advanced form of RV disease in patients with SS and CA may be proposed as a therapeutic option for VT treatment. The combination of advanced strategies of imaging together with EAM should be preferred due to the complex arrhythmogenic substrate to improve efficacy and safety.

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tance.

COMMENTS

Case characteristics

A 63-year-old woman affected by a severe form of systemic sclerosis (SS) with previous dual chamber implantable cardioverter defibrillator implant presented with drug-refractory multiple forms of ventricular tachycardias.

Clinical diagnosis

Identification of aneurismal lesion of the right ventricular (RV) with the critical isthmus from which the five morphologies of ventricular tachyarrhythmias (VTs) arise.

Differential diagnosis

Arrhythmogenic right ventricular dysplasia, myocarditis.

Laboratory diagnosis

Positive Antinuclear Antibodies; metabolic panel and liver function test were within normal limits.

Imaging diagnosis

Intracardiac echocardiography allowed to detect a significant RV dilatation with a complex lesion characterized by thin walls and irregular multiple trabeculae.

Pathological diagnosis

A complete electroanatomical map of the aneurysm showed an area of dense scar surrounded with abnormal electrical activities.

Treatment

Five VTs morphologies were terminated by radiofrequency (RF) delivery at the critical isthmus located at the border of the aneurysm.

Related reports

There is no specific recommendation in VT treatment in SS patients, and the use of CA has been reported anecdotally.

Term explanation

CARTO system is a non fluoroscopic mapping system allowing to accurately determine the location of arrhythmia origin, define cardiac chamber geometry in 3D, delineate areas of anatomic interest.

Experiences and lessons

This is the first case of systemic sclerosis patient with multiple VT morphologies undergoing successful CA guided by an integrated approach aiming at the electro-anatomical characterization of RV cardiomyopathy.

Peer review

This case report describes a case of SS complicated by multiple VTs. This very rare case nicely demonstrates image findings together with important electrophysiologic features in this entity.

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Anaphylactic cardiovascular collapse during hemodialysis: Kounis syndrome in the dialysis room

Andreas Mazarakis, Konstantinos Bardousis, George Almpanis, Ira Mazaraki, Athanasios Ouzounis, Nicholas G Kounis

Andreas Mazarakis, Konstantinos Bardousis, George Almpanis, Ira Mazaraki, Athanasios Ouzounis, Department of Cardiology, "Saint Andrews" State General Hospital, 26504 Patras, Achaia, Greece

Nicholas G Kounis, Department of Medical Sciences, Southwestern Greece Highest Institute of Education and Technology, 26221 Patras, Achaia, Greece

Author contributions: Mazarakis A, Bardousis K, Almpanis G, Mazaraki I and Ouzounis A reviewed the literature, and helped to draft the manuscript; Kounis NG critically revised the manuscript; all authors have read and approved the final manuscript.

Correspondence to: Nicholas G Kounis, MD, PhD, FESC, FACC, FAHA, Department of Medical Sciences, Southwestern Greece Highest Institute of Education and Technology, Queen Olga Square, 7 Aratou Street, 2622 Patras, Achaia, Greece. ngkounis@otenet.gr

Telephone: +30-26-10279579 Fax: +30-26-10279579

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Core tip: This is the first report of Kounis syndrome occurring in the dialysis room in a patient using a new dialysis machine. The apparatus components acting as allergens are incriminated since subsequent hemodialysis sessions with the apparatus used before the anaphylactic reaction were without any sequelae. Materials such as polyurethane, polyamide, polycarbonate, silicon rubber and polypropylene acting as allergens might prove risky in sensitive patients during hemodialysis. Atopic patients should be always interrogated about allergies and patch testing concerning the apparatus components should be performed in such patients.

Mazarakis A, Bardousis K, Almpanis G, Mazaraki I, Ouzounis A, Kounis NG. Anaphylactic cardiovascular collapse during hemodialysis: Kounis syndrome in the dialysis room. *World J Cardiol* 2014; 6(10): 1131-1134 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i10/1131.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i10.1131>

Abstract

Kounis syndrome seems to be not a rare disease but a rarely diagnosed disorder. Multiple causes can join forces and trigger the development of this syndrome. We report the first case of Kounis syndrome manifesting as myocardial infarction with cardiovascular collapse that occurred in the dialysis room following an allergic reaction. The dialysis apparatus material of polyurethane, polyamide, polycarbonate, silicon rubber and polypropylene were incriminated causes. Physicians should be aware of the causality and existence of this disorder in order to achieve early and correct diagnosis and apply the appropriate therapeutic measures.

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Key words: Anaphylactic shock; Dialysis room; Dialysis apparatus; Kounis syndrome; Mast cell degranulation

INTRODUCTION

Kounis syndrome is hypersensitivity-associated acute coronary syndrome manifesting as acute myocardial infarction, coronary spasm or even stent thrombosis^[1]. It is caused by numerous drugs, materials, metals, environmental exposures and conditions associated with mast cell activation. During mast cell activation the released mediators can induce either coronary artery spasm which can progress to acute myocardial infarction or atheromatous plaque erosion or rupture culminating to coronary thrombosis. Kounis syndrome is ubiquitous disease affecting patients of any age, from 2-year-old to octogenarians, involving numerous and continuously increasing causes, with broadening clinical manifestations^[2,3]. The following report concerns of a patient who developed



Figure 1 Electrocardiogram showing complete heart block and ST elevation in the inferior leads during anaphylaxis followed by rapid atrial fibrillation during antihistamine and adrenaline administration.



Figure 2 Coronary arteriogram revealing severe left main disease.

this syndrome during hemodialysis. To our knowledge this is the first case of Kounis syndrome occurring in the dialysis room.

CASE REPORT

A 75-year-old diabetic, hypertensive man having history of coronary artery disease with acute myocardial infarction 12 years ago and stent implantation in the left anterior descending coronary artery and undergoing hemodialysis thrice a week for the last 3 years due to diabetic chronic kidney disease, was transferred to the emergency department after an episode of loss of consciousness accompanied by chest discomfort during dialysis.

He was asymptomatic until he had been connected to the hemodialysis machine in the renal unit for his routine dialysis session. The staff of the unit confirmed changing of the hemodialysis apparatus before the current session with an older brand due to unavailability of the previously used machine. The new apparatus consisted of polyamix membrane, potting material made from polyurethane, housing caps made from polycarbonate, protective plugs made from polypropylene and o-ring made from silicon. Five minutes following the connection with the new dialysis apparatus the patient developed an erythematous rash that covered his trunk and complained of feeling “burning” in his face, chest pain, dyspnea, palpitations and suddenly lost consciousness. A severe anaphylactic reaction associated with Kounis syndrome

probably due to the new dialyzer- was suspected and immediate cardiopulmonary resuscitation was started with chest compressions, antihistamines, hydrocortisone intravenously and adrenaline intramuscular doses of 0.2-0.5 mg (1:1000). His blood pressure was 60/40 mmHg and the electrocardiogram revealed complete heart block with cardiac rate of 40 beats per minute and 5-7mm ST elevation in leads II and III, AVF. Within 3 min the patient was alerted but confused. His blood pressure was raised to 85/60 mmHg, electrocardiogram revealed atrial fibrillation with rapid ventricular response 135 beat per minute and ST elevation 1-2 mm in leads II, III and aVF and ST depression 1 mm in leads I, aVL, V1-V3 (Figure 1). He was then transferred to our coronary care unit for further treatment and evaluation.

Upon arrival to the unit the patient was alert, complaining of mild retrosternal chest pain and Killip class II dyspnea. His blood pressure was 90/50 mmHg, the heart rate was 120 bpm regular and his temperature was 36.0 C. The oxygen saturation, while breathing in the room air was 93%, the electrocardiogram revealed sinus tachycardia with 122 bpm and minimal 0.5-1 mm ST elevation in leads II, III and aVF and 1mm horizontal-downsloping ST depression in leads I, aVL, V1-V6.

Treatment started with 300 mg clopidogrel and 4000IU of low molecular weight heparin. He was not aspirin naïve because of his previous myocardial infarction, so no oral loading dose of aspirin was administered. Peak high sensitivity troponin I levels were 0.350 ng/mL, eosinophils 8%, IgEs were elevated to 170 IU/mL (normal levels < 110 IU/mL) but specific IgEs for the dialysis apparatus were not detected. Transthoracic echocardiographic study showed hypokinesis of basal and mid portions of the infero-posterior wall of the left ventricle with an estimated ejection fraction of 50%. Skin prick tests were not performed on ethical grounds.

The patient remained hemodynamically and electrically stable, asymptomatic and afebrile. Coronary angiography revealed severe stenosis of the left main artery and 70% stenosis of the first segment of right coronary artery and patent previous stent (Figure 2). The patient underwent successful coronary artery bypass surgery. Subsequent hemodialysis sessions with the apparatus used before the anaphylactic reaction were re-started and were without any sequelae.

DISCUSSION

The described patient developed severe anaphylaxis associated with myocardial infarction and cardiovascular collapse in the hemodialysis room soon after changing the dialysis apparatus. He was found to have increased IgEs and eosinophil count suggesting hypersensitivity reaction and was diagnosed as type II variant of Kounis syndrome. All 4 components of the used apparatus namely polyamix membrane, potting material made from polyurethane, housing caps made from polycarbonate, protective plugs made from polypropylene and o-ring made from silicon rubber have been incriminated to induce hypersensitivity reactions. Polyamix membrane is made from a polymer blend of polyarylethersulfone, polyvinylpyrrolidone and polyamide all of which are sensitizers^[4]. Polyurethane chemicals are produced by the reaction of isocyanates and they may cause allergic contact dermatitis or precipitate asthma attacks^[5]. Polycarbonate can induced allergic reactions especially in dental procedures^[6]. Polypropylene is able to induce irritant contact dermatitis^[7] and silicon rubber has induced hypersensitivity reactions known as “latex-fruit syndrome”^[8]. These materials have been incriminated to induce hypersensitivity reactions by activating high and low affinity IgE receptors known as FCγR I, FCγR II, FCεR I and FCεR II receptors situated on both mast cell and platelet surface^[9].

Therefore the described patient was exposed to 5 antigens. Indeed, clinical studies indicate that sensitive patients simultaneously exposed to several allergens can have more symptoms than mono-sensitized individuals^[10]. This could be an explanation for the patient’s immediate cardiovascular collapse. On the other hand, immunoglobulin E (IgE) antibodies with different specificities can have additive effects and small, even sub-threshold numbers of them can join forces and trigger the cells to release their mediators. This can occur when the patient is simultaneously exposed to the corresponding antigens^[11]. The initiation of allergic inflammation takes place when allergens cross-bridge their corresponding, receptor bound, IgE antibodies on the mast cell or basophil cell surface. These cells degranulate and release their mediators when the critical number of bridged IgE antibodies reaches the order of 2000 out of maximal number of some 500000-1000000 IgE antibodies on the cell surface^[12]. A total of approximately 1000 bridges are necessary to induced mast cell degranulation.

Kounis syndrome seems to be not a rare disease but a rarely diagnosed disorder. Multiple and combined causes can trigger the development of this syndrome. Physicians should be aware of its pathophysiology and existence in order to apply predictive, preventive, diagnostic and appropriate therapeutic measures.

COMMENTS

Case characteristics

A 75-year-old diabetic, hypertensive man suffering from coronary artery disease and renal failure developed anaphylactic cardiac collapse soon after been con-

nected with the dialysis apparatus.

Clinical diagnosis

The appearance of erythematous rash that covered his trunk together with feeling “burning” in his face, chest pain, dyspnea, palpitations, sudden loss of consciousness, electrocardiographic changes, increased cardiac enzymes, increased eosinophils and IgEs were suggestive of type II variant of hypersensitivity-associated Kounis syndrome.

Differential diagnosis

The differential diagnosis included anaphylactic shock and acute myocardial infarction but their combination is classical with Kounis acute associated with hypersensitivity coronary syndrome.

Laboratory diagnosis

Serial electrocardiographic changes of complete heart block, atrial fibrillation, ST segment elevation, increased cardiac enzymes and troponin I, increased eosinophils, increased IgEs and hypokinetic basal and mid portions of the infero-posterior wall of the left ventricle were observed.

Imaging diagnosis

Coronary angiography revealed severe stenosis of the left main artery and 70% stenosis of the first segment of right coronary artery but patent previous stent that had been implanted in the left anterior descending artery 12 years previously.

Pathological diagnosis

Neither pathological examination nor skin biopsy for the erythematous rash was thought necessary to be performed.

Treatment

The patient was treated initially with chest compressions, antihistamines, hydrocortisone intravenously and adrenaline intramuscular doses followed by clopidogrel and of low molecular weight heparin and finally underwent successful coronary artery bypass surgery. Subsequent hemodialysis sessions with the apparatus used before the anaphylactic reaction were re-started and were without any sequelae.

Related reports

No related reports are available and this case of Kounis syndrome is the first in the world literature.

Term explanation

This case of Kounis type II variant syndrome is characterized as unique.

Experience and lessons

Kounis syndrome is not a rare disease but a rarely diagnosed disorder caused by multiple and combined causes. Therefore physicians should be aware of its pathophysiology and existence in order to apply predictive, preventive, diagnostic and appropriate therapeutic measures.

Peer review

This is a very interesting report of the case of Kounis syndrome occurring in the dialysis room.

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**TOPIC HIGHLIGHT**

- 1135 Role of microparticles in endothelial dysfunction and arterial hypertension
Helbing T, Olivier C, Bode C, Moser M, Diehl P
- 1140 Bleeding risk stratification in an era of aggressive management of acute coronary syndromes
Abu-Assi E, Raposeiras-Roubin S, García-Acuña JM, González-Juanatey JR
- 1149 Infant with cardiomyopathy: When to suspect inborn errors of metabolism?
Byers SL, Ficicioglu C
- 1156 Importance of genetic evaluation and testing in pediatric cardiomyopathy
Tariq M, Ware SM
- 1166 Diagnosis and management of ischemic cardiomyopathy: Role of cardiovascular magnetic resonance imaging
Doesch C, Papavassiliu T
- 1175 Positive airway pressure therapy for heart failure
Kato T, Suda S, Kasai T
- 1192 Magnetic resonance imaging and multi-detector computed tomography assessment of extracellular compartment in ischemic and non-ischemic myocardial pathologies
Saeed M, Hetts SW, Jablonowski R, Wilson MW

REVIEW

- 1209 Blood glucose management in the patient undergoing cardiac surgery: A review
Reddy P, Duggar B, Butterworth J

MINIREVIEWS

- 1218 Surgical management of moderate ischemic mitral valve regurgitation: Where do we stand?
Fattouch K, Castrovinci S, Murana G, Moscarelli M, Speziale G

CASE REPORT

- 1223 Primary angioplasty for infarction due to isolated right ventricular artery occlusion
Chahal AA, Kim MY, Borg AN, Al-Najjar Y

Contents

World Journal of Cardiology
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APPENDIX I-V Instructions to authors

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Role of microparticles in endothelial dysfunction and arterial hypertension

Thomas Helbing, Christoph Olivier, Christoph Bode, Martin Moser, Philipp Diehl

Thomas Helbing, Christoph Olivier, Christoph Bode, Martin Moser, Philipp Diehl, Department of Cardiology and Angiology I, Heart Center Freiburg University, 79106 Freiburg, Germany

Author contributions: All authors contributed to this paper.
Correspondence to: Philipp Diehl, MD, FESC, Department of Cardiology and Angiology I, Heart Center Freiburg University, Hugstetterstr. 55, 79106 Freiburg,

Germany. philipp.diehl@universitaets-herzzentrum.de
Telephone: +49-761-27034010 Fax: +49-761-27035600

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Abstract

Microparticles are small cell vesicles that can be released by almost all eukaryotic cells during cellular stress and cell activation. Within the last 1-2 decades it has been shown that microparticles are useful blood surrogate markers for different pathological conditions, such as vascular inflammation, coagulation and tumour diseases. Several studies have investigated the abundance of microparticles of different cellular origins in multiple cardiovascular diseases. It thereby has been shown that microparticles released by platelets, leukocytes and endothelial cells can be found in conditions of endothelial dysfunction, acute and chronic vascular inflammation and hypercoagulation. In addition to their function as surrogate markers, several studies indicate that circulating microparticles can fuse with distinct target cells, such as endothelial cells or leukocyte, and thereby deliver cellular components of their parental cells to the target cells. Hence, microparticles are a novel entity of circulating, paracrine, biological vectors which can influence the phenotype, the function and presumably even the transcriptome of their target cells. This review article aims to give a brief overview about the microparticle biology with a focus on endothelial activation and arterial hypertension. More detailed information about the role of microparticles in patho-

physiology and disease can be found in already published work.

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Key words: Microparticles; Arterial hypertension; Endothelial dysfunction; Biological vectors; Inflammation

Core tip: Microparticles are small cell vesicles which can be released from many cells (*e.g.*, endothelial cells, platelets, leukocytes) into circulation and that can be quantified with flow cytometry. Several studies have shown that specific microparticles subtypes are increased in conditions enhanced vascular inflammation and coagulation. Thereby, microparticles have become surrogate markers, which can be used to assess for example leukocyte and endothelial cell activation. Additionally, by fusion with other cells, microparticles transfer cellular components of their parental cells to their target cells, which often results in altered function of the target cells.

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INTRODUCTION

What are microparticles?

During cell activation, multiple eukaryotic cells, such as endothelial cells or leukocytes, but also prokaryotes, have the ability to shed little cell blebs, so called microparticles^[1,2]. Microparticles consist of the cell membrane as well as of the cytoplasm of their maternal cells and can be classified by flow cytometry into for example endothelial microparticles (EMPs), leukocyte microparticles and

platelet microparticles (PMPs). When microparticles were first described by Wolf^[3] over 40 years ago, it was suggested that they are only a kind of cellular debris. However, within the last couple of years microparticles have gained increasing interest in different medical fields and recent effort has been undertaken to investigate the biology of microparticles, as well as the impact of microparticles on different diseases^[4-7]. It thereby has become evident that microparticles can be used as circulating surrogate markers for several pathophysiological conditions, such as inflammation, coagulation but also metastatic diseases and additionally are important circulating biological vectors^[1].

Microparticles as biological vectors in circulation

The biology of microparticles is still incompletely understood, but it is evident that microparticles have far more functions than only activating inflammatory cells and the coagulation cascade. It recently has been shown that they bind to and fuse with distinct target cells, a process that is at least partly mediated by specific interactions of microparticles with surface receptors (such as Mac-1) of the target cell^[8]. By fusion with their target cells, microparticles deliver cytoplasm as well as membrane anchored surface receptors to their destination cells. This process is frequently associated with changes of the target cells phenotype and function. Hence, microparticles are an own kind of biological vectors modulating the function of their target cells remote from the location where they initially had been released.

Elevated microparticle levels can often be found in pathological conditions which are associated with cell mediated inflammation and coagulation. To assess the inflammatory effect of platelet microparticles, which is the largest microparticle fraction in the blood, Jy *et al*^[9] investigated the effect of PMPs on neutrophils. They found that microparticles released from platelets attach to neutrophils and activate those. Hence, platelet microparticles may in fact be an additional link between vascular coagulation and inflammation in cardiovascular disease.

Hypothesizing that microparticles might not only influence the phenotype but also the transcriptome of their target cells, Hunter *et al*^[10] assessed whether microparticles from mononuclear cells contain microRNAs, which are small non-coding RNA molecules that regulate mRNA translation and thereby affect post transcriptional gene expression. In this ground breaking study, they found that microparticles indeed contain a broad spectrum of different microRNAs, which they might deliver to their target cells and presumably affect the target cells protein synthesis. Interestingly, when compared to microRNA patterns of their cells of origin, microparticles do not contain a random set of microRNAs of their paternal cells, but are loaded with a distinct, specific selection of miRNAs^[11]. These findings suggest that microparticle release is a highly regulated process in which cell vesicles are "loaded" from their cells of origin with specific RNA molecules which might eventually be transferred to their target cells. However, to date the underlying molecular

mechanisms of this loading process are not understood.

To what extent circulating microparticles are involved in intercellular signalling was demonstrated by a pivotal study of Janowska-Wieczorek *et al*^[12]. They found that PMPs transfer the platelet surface receptor glycoprotein (GP) II b/III a to the surfaces of different lung cancer cell lines. As the GP II b/III a integrin has a high affinity to (sub)endothelial antigens, tumour cells that were pre-incubated with platelet microparticles also showed increased metastasization. Hence, PMPs might be directly involved in the progression of tumour diseases.

In summary, microparticles are small cell blebs that represent a novel way of intercellular communication, which seems to be particularly relevant for inflammatory and pro-coagulatory diseases. Due to the effects on their target cells, microparticles are able to change the phenotype, the function and presumably also the transcriptome of their target cells and might be involved in the pathogenesis of several cardiovascular diseases^[1].

ARTERIAL HYPERTENSION

Arterial hypertension is a strong risk factor for atherosclerosis and vascular mortality and often starts with endothelial dysfunction^[13,14]. Early diagnosis of impaired endothelial function is crucial to allow medical anti-inflammatory, endothelium-protective treatment at an early disease stage. Reflecting endothelial dysfunction, endothelial microparticles might be a valuable tool to assess endothelial dysfunction, particularly in asymptomatic patients.

Microparticles in endothelial dysfunction and arterial hypertension

Arterial hypertension is a multifactor disease that is strongly promoted by endothelial dysfunction^[15,16]. Recent data indicate that altered, activated endothelial cells release endothelial microparticles into circulation. EMPs can be used as cellular surrogate markers for endothelial dysfunction and are increased in several diseases with an altered endothelial function, such as atherosclerosis, aortic valve stenosis and pulmonary hypertension^[17-20]. It recently was published that endothelial microparticles are even associated with several cardiovascular risk factors in the Framingham Heart Study^[21].

However, besides their role as surrogate markers, microparticles are furthermore involved in the progression of impaired endothelial function as well as in angiogenesis^[22,23]. For example Burger *et al*^[24] assessed the effect of microparticles on endothelial inflammation and found that microparticles themselves induce endothelial expression of vascular cell adhesion molecule 1, platelet endothelial cell adhesion molecule and adhesion of J774A.1 cells, which is a cell line with macrophage characteristics^[24]. Along the same line of evidence, Boulanger *et al*^[25] investigated the mechanisms how microparticles induce endothelial dysfunction and found that MPs from patients with myocardial infarction, but not from healthy

controls, induced endothelial dysfunction by impairing the endothelial nitric oxide transduction pathway. These data were confirmed by Martin *et al*^[26] who discovered that T cell microparticles reduced endothelial nitric oxide- and prostacyclin mediated vasodilatation and decreased expression levels of endothelial nitric oxide synthase.

One of the few studies investigating the interconnection between microparticles and arterial hypertension was performed by Preston *et al*^[20]. They assessed the abundance of endothelial microparticles in patients with untreated severe hypertension *vs* those with mild hypertension compared to normotensive individuals. It was found that microparticles released from endothelial cells and platelets were significantly increased in patients with severe arterial hypertension and that endothelial microparticles correlated strongly with the level of both systolic and diastolic blood pressures. Thus, it can be suggested that EMPs and PMPs can be used as circulating markers for endothelial injury in arterial hypertension. The findings described by Preston *et al*^[20] are supported by studies, in which increased levels of circulating endothelial microparticles had been found in patients with pre-eclampsia, a disease that is characterized by vascular inflammation, altered endothelial function and arterial hypertension^[27,28].

The Renin Angiotensin System (RAS) plays a key role in arterial hypertension and is the target for anti-hypertensive medical treatment. It has been supposed that angiotensin II, which is the final effector of the RAS, not only affects the blood pressure but furthermore induces a pro-thrombotic state. Hypothesizing that the RAS might be involved in the generation of pro-thrombotic microparticles, Cordazzo *et al*^[29] investigated the effect of angiotensin II on the release of microparticles from mononuclear cells. They found that angiotensin II indeed induces shedding of pro-thrombotic MP from mononuclear cells. The data of Cordazzo support the suggestion that microparticles might in fact be the link between the activation of the renin angiotensin system and a pro-thrombotic state, which can be found in patients suffering from arterial hypertension.

End-organ damage, such as hypertensive nephropathy with impaired kidney function, is a common complication of patients with arterial hypertension. To assess whether endothelial microparticles might be involved in impaired renal function under arterial hypertension, Hsu *et al*^[30] measured endothelial microparticles, endothelial progenitor cells (EPCs) and the glomerular filtration rate in patients suffering from arterial hypertension. They found that elevated EMPs to EPCs ratios are associated with a decline of the glomerular filtration rate in hypertensive patients. These data underline the impact of endothelial damage assessed by the EMP to EPC ratio on the progression of impaired kidney functions in arterial hypertensive patients.

In conclusion, particularly endothelial microparticles can be found in several conditions that are associated with arterial hypertension. EMPs are not only valuable

surrogate markers reflecting the extent of endothelial cell dysfunction but additionally might promote the progression of arterial hypertension and its complications.

WHAT BRINGS THE FUTURE?

Microparticles are promising surrogate markers for a variety of pathological conditions, particularly in conditions that are associated with impaired endothelial function and arterial hypertension (Table 1). However, a lack of standardization of microparticle definitions and methods used to quantify microparticles makes it difficult to compare results from different research groups. As microparticles have a highly complex molecular architecture, they are more fragile than for example blood proteins, which are often used as clinical surrogate parameters. Hence, the way how blood samples for microparticle measurements are taken, such as the diameter and the length of the needle that was used, is critical and can significantly influence flow cytometric analysis of microparticles. Finally, even technical characteristics of the flow cytometry used to analysis microparticles can influence measurement results. Therefore, the International Society on Thrombosis and Haemostasis (www.isth.org) and the International Society for Extracellular Vesicles (<http://www.isev.org>) are working on recommendations for standardized protocols for microparticle measurements. Standardized studies will need to assess the diagnostic value of microparticles as surrogate markers in arterial hypertension.

As microparticles reflect a variety of different pathological changes in the vascular system (*e.g.*, inflammation, coagulation, activation of different cell types, *etc.*) they might represent a broader spectrum of cellular changes in circulation than measuring only one distinct soluble marker protein. Furthermore, besides their role as vascular surrogate markers, microparticle measurement can presumably be used to monitor the success of medical treatments of diseases that are associated with vascular inflammation. However, large clinical multicentre studies are necessary to assess whether microparticles of different cellular origin can be used as surrogate markers and as tools for drug monitoring in different cardiovascular diseases.

Until now, only very few studies have investigated the effect of different drugs on circulating microparticles. Nomura *et al*^[31] found that eicosapentaenoic acid, which is an omega-3 fatty acid, reduces endothelial derived microparticles in patients suffering from type 2 diabetes. Tramontano *et al*^[32] described that fluvastatin has a protective effect on endothelial cells and inhibits EMP release and Morel *et al*^[33] reports that vitamin C reduces endothelial and platelet derived microparticles in patients with myocardial infarction. Even if these data are promising, their results need to be confirmed by randomized multicentre studies and it needs to be assessed whether a reduction of microparticle levels is associated with a beneficial patient outcome.

In conclusion, microparticles are small cell vesicles re-

Table 1 Overview about studies investigating the interrelation between microparticles and endothelial dysfunction/arterial hypertension

Study subjects	Flow cytometric MP characteristics	Findings	Ref.
Framingham offspring cohort	CD144 ⁺ CD31 ⁺ /CD41 ⁻	Increased CD144 ⁺ MP correlate with Arterial hypertension Elevated triglycerides Metabolic syndrome Increased CD31 ⁺ /CD41 ⁻ correlate with elevated triglycerides	Amabile <i>et al</i> ^[21]
MPs of AMI patients	Isolated blood MPs	MPs from AMI patients impair the endothelial nitric oxide pathway	Boulanger <i>et al</i> ^[25]
Ang II stimulated mouse aortic endothelial cells	Annexin V ⁺	Ang II induces EMP release	Burger <i>et al</i> ^[24]
(Microparticles of) human mononuclear cells	CD144 ⁺ Microparticles from mononuclear cells	EMPs increase endothelial expression of VCAM-1, PCAM and adhesion of J774A.1 cells Ang II induces MP release of mononuclear cells	Cordazzo <i>et al</i> ^[29]
MPs of human lymphoid CEM T cell line	Isolated cell culture MPs	Angiotensin receptor type 2 inhibitors reduce Ang II induced MP release MPs decrease expression levels of eNOS	Martin <i>et al</i> ^[26]
EMPs of women with pre-eclampsia	CD62E ⁺ CD31 ⁺ /42b ⁻	MPs induce endothelial dysfunction by altering the NO- and prostacyclin pathways Women with preeclampsia have higher EMP levels than those with gestational hypertension and controls	González-Quintero <i>et al</i> ^[28]
MPs levels of patients with art Hypertension	CD31 ⁺ /CD42 ⁺ CD41 ⁺	Increased EMPs and PMPs in patients with severe arterial hypertension EMPs and PMPs levels correlate with blood pressure	Preston <i>et al</i> ^[20]

EMPs: Example endothelial microparticles; PMPs: Platelet microparticles; Ang II: Angiotensin II; MPs: Microparticles; eNOS: Endothelial nitric oxide synthase; VCAM-1: Vascular cell adhesion molecule 1; PCAM: Platelet endothelial cell adhesion molecule; AMI: Acute myocardial infarction; NO: Nitric oxide.

leased by a huge variety of cells reflecting the state of activation of their parental cells. Besides functioning as surrogate markers for example for endothelial dysfunction, recent evidence indicates that they additionally influence the progression of several cardiovascular diseases. Hence, circulating microparticles might not only be valuable surrogate markers for different pathological conditions but furthermore be novel therapeutic targets by which the progression of microparticle mediated diseases might be influenced.

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Bleeding risk stratification in an era of aggressive management of acute coronary syndromes

Emad Abu-Assi, Sergio Raposeiras-Roubín, José María García-Acuña, José Ramón González-Juanatey

Emad Abu-Assi, Sergio Raposeiras-Roubín, José María García-Acuña, José Ramón González-Juanatey, Department of Cardiology, University Clinical Hospital of Santiago de Compostela, 15071 Santiago de Compostela, Spain

Author contributions: Abu-Assi E coordinated the development of the paper, and wrote the comparison of bleeding risk scores; Raposeiras-Roubín S wrote the quantitative evaluation of bleeding risk; García-Acuña JM wrote the long-term bleeding risk stratification and prognostic implications; González-Juanatey JR wrote the introduction and bleeding definition; all the authors revised the final manuscript.

Correspondence to: Emad Abu-Assi, MD, PhD, Department of Cardiology, University Clinical Hospital of Santiago de Compostela, Travesía Choupana s/n 15706, 15071 Santiago de Compostela, Spain. eabuassi@yahoo.es

Telephone: +34-981-950000 Fax: +34-981-959750

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Abstract

Major bleeding is currently one of the most common non-cardiac complications observed in the treatment of patients with acute coronary syndrome (ACS). Hemorrhagic complications occur with a frequency of 1% to 10% during treatment for ACS. In fact, bleeding events are the most common extrinsic complication associated with ACS therapy. The identification of clinical characteristics and particularities of the antithrombin therapy associated with an increased risk of hemorrhagic complications would make it possible to adopt prevention strategies, especially among those exposed to greater risk. The international societies of cardiology renewed emphasis on bleeding risk stratification in order to decide strategy and therapy for patients with ACS. With this review, we performed an update about the ACS bleeding risk scores most frequently used in daily clinical practice.

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Key words: Bleeding; Acute coronary syndrome; Risk scores; Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA guidelines; Acute Coronary Treatment and Intervention Outcomes Network

Core tip: Bleeding is the main non-thrombotic complication associated with acute coronary syndrome. Bleeding implies a worse prognosis due itself directly (fatal bleeding, for example, intracranial bleeding) and indirectly (discontinuation of antithrombotic therapy). For this it is important to do an adequate bleeding risk stratification in all patients with acute coronary syndrome. In this review we analyze the different risk factors for bleeding, along with the bleeding risk scores currently available.

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INTRODUCTION

The classic aim of acute coronary syndrome (ACS) therapy was to reduce mortality and to prevent or minimize ischemic complications. This was possible with percutaneous coronary intervention and with antithrombotic drugs^[1]; however, these therapies have led to an increased risk of bleeding complications^[2]. Until the recent past, bleeding was thought to be inherent to the modern therapeutic approach in ACS and percutaneous coronary intervention (PCI)^[3]. Nowadays this consideration has been changed. Clinical trials have demonstrated that major bleeding has a strong impact on the risk of death,

myocardial infarction and stroke in patients with ACS^[4]. Therefore, a reduction in bleeding events translates into improved survival^[1]. Because today we have a large arsenal of antiplatelet and anticoagulant drugs with different profile of efficacy and safety, it is important to make a proper selection of medication in order to balance the ischemic and hemorrhagic risk^[5-8]. European and American Societies of Cardiology recommend bleeding risk stratification to guide ACS treatment^[9-12].

INCIDENCE OF BLEEDING: THE PROBLEM OF THE DEFINITION

Hemorrhagic complications occur with a frequency of 1% to 10% during treatment for ACS and after PCI^[13]. The National Cardiovascular Data Registry Acute Coronary Treatment and Intervention Outcomes Network Registry Get with the Guidelines (NCDR ACTION Registry-GWTG)^[14] evaluated 72699 patients with non ST-segment elevation myocardial infarction (NSTEMI) and 48943 with ST-segment elevation myocardial infarction (STEMI). The reported major bleeding rate was approximately 9% among patients with NSTEMI and 12% among those patients with STEMI. Of note, the bleeding rates were significantly influenced by the presence of comorbidities, as well as by the use of invasive strategies in both NSTEMI and STEMI.

Bleeding rates depend mainly on the clinical setting and on the definition of bleeding events^[15,16]. Since their initial development, both TIMI and GUSTO criteria have been applied to identify very significant bleeding in a wide range of clinical trials^[17,18], but a myriad of other criteria have also been created^[19] [the CURE^[20], Randomized Evaluation of PCI Linking Angiomax to Reduced Clinical Events (REPLACE)^[21], STEEPLE^[22], OASIS^[6] and acute catheterization and urgent intervention triage strategy (ACUITY)^[8] bleeding definitions] (Table 1).

The Bleeding Academic Research Consortium (BARC) convened in 2010 was idealized with the intention of reviewing the existing definitions and developing standards for the analysis of hemorrhagic complications^[13]. Among the recommendations of the panel, the consensus around the challenge of creating a single definition of major bleeding to be adopted stands out since the analyzed population is extremely variable as to its characteristics, clinical profile, follow-up time length, and due to the constant temporary modifications in clinical therapy and treatment strategies considered appropriate at its time. Basing on this, BARC participants proposed 5 bleeding types (Table 1)^[6].

PREDISPOSING FACTORS

Major bleeding is currently one of the most common non-cardiac complications observed in the treatment of ACS patients. The identification of clinical characteristics and particularities of the antithrombin therapy associated with an increased risk of hemorrhagic complications

would make it possible to adopt prevention strategies, especially among those exposed to greater risk^[15].

In this way, different studies exposed the main predictors of major bleeding in the treatment of ACS. The Global Registry of Acute Coronary Events (GRACE) investigators developed a risk score of major bleeding, basing on a registry with 24045 ACS patients, of which 933 (3.9%) developed an episode of major bleeding during hospitalization^[23]. They identified 7 independent predictors of bleeding: age, female gender, prior bleeding, kidney dysfunction, fibrinolysis, glycoprotein II b/IIIa inhibitors (GPI) use, and PCI. The most frequent bleeding sites were gastrointestinal (31.5%) and those related to the vascular access site (23.8%).

In the ACUITY study^[22], authors identified 8 variables related to greater risk of bleeding were identified: female sex, anemia, advanced age, use of unfractionated heparin and II b/IIIa inhibitors instead of isolated bivalirudin, elevated serum creatinine, increased leukocyte count, absence of previous PCI, prior stroke, ST-segment elevation ≥ 1 mm, and routine use of GPI.

In an analysis of the Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA guidelines (CRUSADE) database^[1], with 89134 high-risk NSTEMI patients and with a incidence of major bleeding of 9.4%, 8 variables were identified as independent predictors of major bleeding: female sex, peripheral vascular disease, diabetes, systolic blood pressure, heart rate, congestive heart failure, creatinine clearance, and hematocrit.

In the REPLACE registry, female sex, anemia, and glomerular filtrate rate were also identified as independent predictor of bleeding^[24]. Age > 55 years, low molecular weight heparin within 48 h pre-PCI, GPI, and intra-aortic balloon pump use were the other clinical variables associated with higher rate of major bleeding in the REPLACE trial.

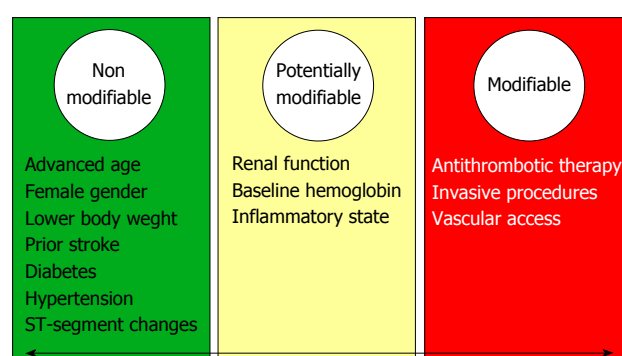
In a global way, bleeding risk factors can be categorized into nonmodifiable and modifiable groups^[25]. Commonly reported bleeding risk factors in patients with ACS are summarized in Figure 1.

According to the non-modifiable risk factors it is important to remarked 2 clinical variables: advanced age and female sex. Advanced age would predispose to a greater risk of bleeding due to injuries located in the vessels and systemic diffuse vessel disease. In the GRACE registry encompassing the whole spectrum of ACS, the adjusted odds of having a major hemorrhage prior to discharge increased by about 30% per decade of age (OR = 1.28, 95%CI: 1.21-1.37)^[6,23,26]. In relation to female sex, within the GRACE registry, women had a 43% higher likelihood of developing major bleeds in-hospital compared with men (adjusted OR = 1.43, 95%CI: 1.23-1.66)^[6,23]. It is believed that the smaller body size as well as the lower vessel size, reduced creatinine clearance, higher prevalence of comorbidities, higher risk of drug overdosing, older age at the moment of admission, and a lower threshold for transfusion due to lower baseline levels of hemoglobin would justify the relationship between female sex and

Table 1 Bleeding definitions

Trial	Definition
TIMI	Major bleeding: Intracranial hemorrhage or decrease of 5 g/dL in hemoglobin or 15% in hematocrit Minor bleeding: Decrease of 3 g/dL in hemoglobin with known source of blood loss or decrease of 4 g/dL in hemoglobin without known source of blood loss
GUSTO	Major bleeding: Fatal, intracranial, Retroperitoneal, intraocular leading to vision loss, or transfusion of 2 U Minor bleeding: any clinically significant bleeding not meeting major criteria leading to study drug interruption, surgery, or transfusion of 1 U of blood
ACUTY	Major bleeding: Intracranial or intraocular bleeding, hemorrhage at access site requiring intervention, hematoma ≥ 5 cm, decrease ≥ 4 g/dL of hemoglobin without overt bleeding source or ≥ 3 g/dL with source, reoperation for bleeding, or transfusion of blood product Minor bleeding: any clinically significant bleeding not meeting major criteria
CRUSADE	Major bleeding: intracranial hemorrhage, documented retroperitoneal bleed, hematocrit drop $\geq 12\%$ (baseline to nadir), any red blood cell transfusion when baseline hematocrit was $\geq 28\%$, or any red blood cell transfusion when baseline hematocrit was $< 28\%$ with witnessed bleed Minor bleeding: any clinically significant bleeding not meeting major criteria
GRACE	Major bleeding: Life-threatening bleeding requiring transfusion of ≥ 2 U of packed red blood cells, bleeding resulting in absolute hematocrit decrease $\geq 10\%$ or death hemorrhagic/subdural hematoma Minor bleeding: any clinically significant bleeding not meeting major criteria
BARC	Type 0: No bleeding Type 1: Bleeding that is not actionable and does not cause the patient to seek unscheduled performance of studies, hospitalization, or treatment by a health care professional; may include episodes leading to self-discontinuation of medical therapy by the patient without consulting a health care professional Type 2: Any overt, actionable sign of bleeding (<i>e.g.</i> , more bleeding than would be expected for a clinical circumstance, including bleeding found by imaging alone) that does not fit the criteria for type 3, 4, or 5 but does meet at least one of the following criteria: requiring nonsurgical, medical intervention by a health care professional; leading to hospitalization or increased level of care; or prompting evaluation Type 3a: Overt bleeding plus hemoglobin drop of 3-5 g/dL (provided hemoglobin drop is related to bleed), or any transfusion with overt bleeding Type 3b: Overt bleeding plus hemoglobin drop ≥ 5 g/dL (provided hemoglobin drop is related to bleed), or cardiac tamponade, or bleeding requiring surgical intervention for control (excluding dental/nasal/skin/hemorrhoid), or bleeding requiring intravenous vasoactive agents Type 3c: Intracranial bleeding (does not include microbleeds or hemorrhagic transformation, does include intraspinal), or subcategories confirmed by autopsy or imaging or lumbar puncture, or intraocular bleed compromising vision Type 4: Coronary artery bypass graft-related bleeding, or perioperative intracranial bleeding within 48 h, or reoperation after closure of sternotomy for the purpose of controlling bleeding, or transfusion of ≥ 5 U whole blood or packed red blood cells within a 48-h period, or chest tube output ≥ 2 L within a 24-h period Type 5 or fatal bleeding A: Probable fatal bleeding; no autopsy or imaging confirmation but clinically suspicious Type 5 or fatal bleeding B: Definite fatal bleeding; overt bleeding or autopsy or imaging confirmation

ACUTY: Acute catheterization and urgent intervention triage strategy; CRUSADE: Can Rapid risk stratification of Unstable angina patients Suppress AD-verse outcomes with Early implementation of the ACC/AHA guidelines; GRACE: Global Registry of Acute Coronary Events; BARC: Bleeding Academic Research Consortium.

**Figure 1** Bleeding risk factors in patients with acute coronary syndrome.

bleeding^[15].

In relation with potentially modifiable factors, renal function is the most interesting. Santopinto *et al.*^[27] demonstrated that patients with moderate renal dysfunction were twice as likely to die (OR = 2.09, 95%CI: 1.55-2.81) and those patients with severe renal dysfunction are almost four times more likely to die (OR = 3.71, 95%CI:

2.57-5.37)^[27]. Other potentially modifiable variable, with great interest in last years, is body mass index (BMI)^[28]. Several epidemiologic studies have demonstrated that higher BMI was inversely associated with lower risk of mortality among patients with coronary artery disease (obesity-mortality paradox). As we know, the association between short-term death and BMI was affected not only by the ischemic risk but also by the major bleeding risk^[25]. Recently, a meta-analysis have clarified the relationship between the risk of bleeding and BMI following PCI^[29]. In this study, it was concluded that class I / II obese patients had the lowest risk of bleedings.

With regard to modifiable risk factors, two variables deserve a special mention: antithrombotic therapy and vascular access. Antithrombotic therapy would be influenced by pharmacodynamic and pharmacokinetic characteristics of the antithrombotic agents^[15]. In this way, we can exemplify with the differences between fondaparinux, bivalirudin and enoxaparin, or the differences between clopidogrel, prasugrel, and ticagrelor. Fondaparinux and bivalirudin showed to reduce the rate of bleeding compli-

cations when compared with low molecular weight heparin and heparin sodium, with adequate antithrombotic ability (although fondaparinux have a slightly increased risk of catheter thrombosis in patients undergoing PCI). A critical aspect in the appropriate use of anticoagulant agents is dose adjustment according to the renal function. Current guidelines indicate dose reduction of enoxaparin to 1 mg/kg once daily in the case of severe renal failure (CrCl < 30 mL/min), and consider monitoring of anti-Xa activity^[9,12]. Fondaparinux is contraindicated in severe renal failure (CrCl < 20 mL/min), and is considered the drug of choice in patients with moderately reduced renal function (CrCl 30-60 mL/min). Regarding bivalirudin, patients with moderate renal impairment (30-59 mL/min) should receive an infusion of 1.75 mg/kg per hour, or 1 mg/kg per hour if the creatinine clearance is < 30 mL/min (0.25 mg/kg per hour if the patient is on haemodialysis). In presence of CrCl < 30 mL/min or eGFR is < 30 mL/min per 1.73 m², unfractionated heparin infusion adjusted to activated partial thromboplastin time is the recommended anticoagulant, albeit fondaparinux could be maintained until CrCl < 20 mL/min.

For the vascular access, the use of radial access significantly reduces bleeding complications in PCI compared with femoral access^[30]. Importantly, vascular closure devices should be used in patients without significant arterial calcification in order to obtain satisfactory results.

In addition to these clinical factors, current research is focused on meeting new bleeding risk indicators. In this sense, genetic factors have been associated to bleeding^[26]. For example, in clopidogrel-treated patients, the gain-function variant CYP2C19*17 was associated with higher bleeding rate^[6,31]. This is an area with great projection in the near future.

QUANTITATIVE EVALUATION OF BLEEDING RISK

The contemporary cardiology walks towards those predictive models that minimize as much as possible to morbidity and mortality resulting from cardiovascular disease^[32]. This is to minimize the subjective component of clinical evaluation of a given patient. Therefore risk stratification is that characterizes modern clinical cardiologist^[33]. Since patient's admission, there are many factors that determine the patient's prognosis in terms of mortality and morbidity. In this way, is necessary to go reassessing the patient risk at all times. Regarding acute coronary syndrome patient, particularly in relation to bleeding, there are a lot of variables that determine the hemorrhagic risk. The interaction between these variables is not easy to assess clinically. This is where lies the advantage of risk scores, which enable integration of all these variables providing a measure of risk that would not be possible otherwise. And this is the reason because of objective risk assessment provides superior risk discrimination when compared with physician-estimated risk^[34]. Although there are several bleeding risk scores,

there is no consensus about what is the best for bleeding risk assessment in daily clinical practice.

Contemporary bleeding risk scores (RS) (Table 2) in ACS comprise: REPLACE^[24], CRUSADE^[11], ACTION^[35], and that derived by Mehran *et al*^[36] from the combined dataset of ACUITY/HORIZONS-AMI trials. The CRUSADE risk score was developed to assess the in-hospital bleeding risk help during NSTEMI, whereas the ACTION and Mehran *et al*^[36] models were derived from NSTEMI and STEMI patients. In addition to these risk models, the REPLACE proposes a stratification of the bleeding risk for patients submitted to PCI through femoral Access.

ADOPTION OF BLEEDING RISK SCORES

REPLACE

Using data from the multicenter studies REPLACE-1 e 2^[37,38], Nikolsky *et al*^[24] proposed a bleeding RS for patients submitted to PCI through femoral access (www.bleedingriskscore.org). In multivariate analysis performed in 5395 patients, seven variables were identified as predictors of major bleeding: age, female sex, chronic kidney dysfunction, anemia, use of low-molecular-weight heparin, administration of GPI, and the use of intra-aortic balloon pump. Based on them, a risk score was constructed, with an adequate discrimination (C-statistic = 0.62). The main limitation is that this risk score was derived from a highly selective population undergoing PCI using the femoral approach.

CRUSADE

More recently, investigators of the CRUSADE registry developed and validated a risk stratification tool for in-hospital major bleeding among NSTEMI patients^[11]. Having a database constituted by 89134 patients, within 485 North American hospitals, the authors developed a bleeding risk score with those variables that resulted independent predictors of major bleeding: female sex, diabetes mellitus, peripheral artery disease, heart rate, systolic blood pressure, congestive heart failure, hematocrit, and creatinine clearance (www.crusadebleedingscore.org). Considering only the variables present at admission, the CRUSADE bleeding score is presented as an easily applicable and useful tool in predicting patient risk, in addition to the analysis of the risk of ischemic events, allowing a tailored therapeutic strategy, adapted to the individualized risk profile. Moreover, CRUSADE bleeding risk score was externally validated by Abu-Assi *et al*^[39]. The CRUSADE score showed adequate calibration and excellent discriminatory powerful in the whole population and in the different treatment subgroups, except in patients treated with ≥ 2 antithrombotics who did not undergo cardiac catheterization (C-index = 0.56).

Mehran *et al*^[36] bleeding risk score

Mehran *et al*^[36] using data from the ACUITY and the HORIZONS-AMI trials (17421 patients) developed a bleeding risk score. Six independent baseline predictors

Table 2 Bleeding risk scores

Bleeding risk scores variables	Action		Mehran <i>et al</i>		CRUSADE	
	Values	Points	Values	Points	Values	Points
Sex	Male	0	Male	0	Male	0
	Female	4	Female	8	Female	8
Age (yr)	≤ 40	0	< 50	0		
	41-50	1	50-59	3		
	51-60	2	60-69	6		
	61-70	3	70-79	9		
	71-80	4	≥ 80	12		
	81-90	5				
	≥ 91	6				
Weight (kg)	≤ 50	5				
	51-70	4				
	71-100	3				
	101-120	2				
	121-140	1				
	≥ 141	0				
Systolic blood pressure (mmHg)	≤ 90	4			≤ 90	10
	91-100	3			91-100	8
	101-120	2			101-120	5
	121-140	1			121-180	1
	141-170	0			181-200	3
	171-200	1			≥ 201	5
	≥ 201	2				
Heart rate (BPM)	≤ 40	0			≤ 70	0
	41-60	2			71-80	1
	61-70	3			81-90	3
	71-80	5			91-100	6
	81-100	6			101-110	8
	101-110	8			111-120	10
	111-120	9			≥ 121	11
	121-130	11				
	131-150	12				
	≥ 151	14				
Signs of heart failure	None	0			No	0
	Killip 2-3	3			Yes	7
	Cardiogenic shock	15				
Diabetes mellitus	No	0			No	0
	Yes	3			Yes	6
Prior vascular disease	No	0			No	0
	Yes	3			Yes	6
Home warfarin use	No	0				
	Yes	2				
Antithrombotic medications			Heparin plus GPI	0		
			Bivalirudin	-5		
ECG changes	No ST changes	0	No ST elevation	0		
	ST depresión	3	ST elevation	6		
	ST transient elevation	7				
	ST elevation					
Troponine I			Normal	0		
			Raised	6		
Serum creatinine (mg/ dL)	< 0.80	0	< 1.00	0		
	0.80-1.59	1	1.00-1.19	2		
	1.60-1.99	2	1.20-1.39	3		
	2.00-2.99	4	1.40-1.59	5		
	3.00-3.99	6	1.60-1.79	6		
	4.00-4.99	8	1.80-1.99	8		
	5.00-5.99	10	≥ 2.00	10		
	≥ 6.00	11				
	On dialysis	11				
Creatinine clearance (mL/ min)					≤ 15.0	39
					15.1-30.0	35
					30.1-60.0	28
					60.1-90.0	17
					90.1-120.0	7
					> 120	0
Baseline hemoglobin (g/ dL)	< 5.0	17				
	5.0-7.9	15				

	8.0-9.9	13		
	10.0-10.9	12		
	11.0-13.9	9		
	14.0-15.9	6		
	≥ 16.0	2		
Baseline hematocrit (%)			< 31.0	9
			31.0-33.9	7
			34.0-36.9	3
			37.0-39.9	2
			≥ 40.0	0
Anemia	No	0		
	Yes	6		
White blood cell count (giga/L)	< 10.0	0		
	10.0-11.9	2		
	12.0-13.9	3		
	14.0-15.9	5		
	16.0-17.9	6		
	18.0-19.9	8		
	≥ 20.0	10		

CRUSADE: The Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA guidelines; GPI: Glycoprotein IIb/IIIa inhibitors; ECG: Electrocardiography; BPM: Beats per minute.

for major bleeding were identified: female sex, age, creatinine, white blood cell count, anemia, ST-segment-elevation. The risk score differentiated patients with a 30-d rate of non-CABG-related major bleeding ranging from 1% to over 40%. As a difference with the other bleeding risk scores, this one includes white blood cell count as a risk factor for major bleeding.

ACTION

Using data from the ACTION trial in patients with STEMI and NSTEMI, an in-hospital bleeding risk score was developed^[35]. Twenty-two clinically variables were incorporated into the final regression model: heart rate, baseline hemoglobin, female gender, serum creatinine, age, electrocardiographic changes, heart failure or shock, diabetes, peripheral artery disease, body weight, systolic blood pressure, and home warfarin use. The rate of major bleeding in the overall population was 10.8%. The risk model discriminated well in the derivation (C-statistic = 0.73) and validation (C-statistic = 0.71) cohorts, with an optimal risk gradient: very low risk (3.9% of bleeding), low risk (7.3%), moderate risk (16.1%), high risk (29.0%), and very high risk (39.8%).

COMPARISON OF BLEEDING RISK SCORES

As we have shown above, all bleeding RS have shown good discrimination and calibration. The question is: Which RS should be recommended for the management of patients with ACS? Perhaps for that question we first must be sure about the reliability of a given predictive model in our population. The REPLACE bleeding RS was designed for a femoral approach, so now, in times of radial access, its usefulness is less. The other 3 scores (CRUSADE, ACTION and Mehran *et al*^[36]) were compared recently by the group of Abu Assi on a patient population with ACS (STEMI and NSTEMI)^[40], be-

ing the greatest accuracy obtained with the CRUSADE method, even in patients with STEMI.

Although any score cannot replace the clinical evaluation, data from our study suggests that CRUSADE score represents an useful objective clinical tool which could lead to improvements in ACS care^[40].

LONG-TERM BLEEDING RISK STRATIFICATION

The risk of developing bleeding complications continues after discharge. About 5% of patients develop bleeding complications throughout the first year after hospital discharge being on dual antiplatelet therapy (DAPT)^[41]. There is no risk model to estimate risk of bleeding after discharge in ACS patients. Using data from the REACH registry^[42], a risk score was built although in a stable scenario (not in the ACS setting). Because the CRUSADE risk score performed well among patients taking DAPT, this risk model may be used for bleeding risk stratification in ACS on DAPT after hospital discharge.

PROGNOSTIC IMPLICATIONS OF BLEEDING RISK STRATIFICATION

The main clinical implication of RS is to pave the way for a decision concerning the best antithrombotic strategy to be used aiding individual evaluation for risk of ischemic or hemorrhagic events.

Collectively, innumerable studies have shown a robust association between the occurrence of major bleeding and the necessity of blood cell transfusion with greater mortality in patients admitted with ACS or submitted to PCI (Figure 2). Subherwal *et al*^[1] demonstrated an association between bleeding and in-hospital mortality. Mehran *et al*^[36] showed that major bleeding was an independent predictor of a 3.2-fold increase in mortality.

Although it is coherent to justify the association be-

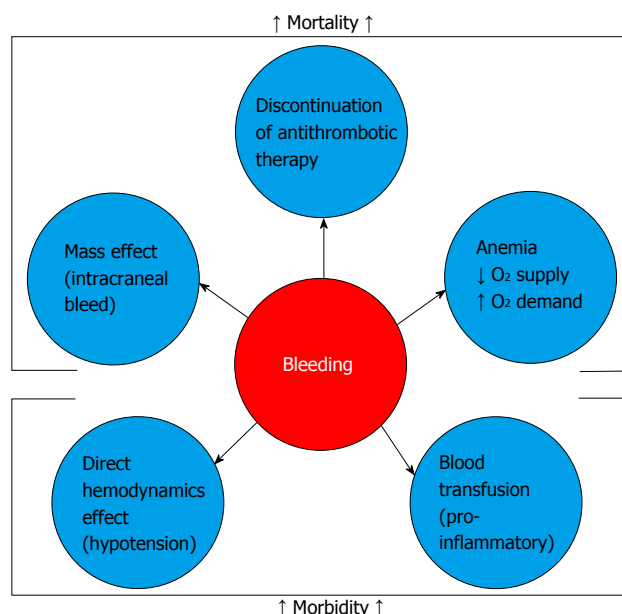


Figure 2 Different links between bleeding and morbidity and mortality in patients with acute coronary syndrome.

tween major bleeding and mortality by the coexistence of comorbidities and risk factors in the population common to the occurrence of these outcomes, today an accumulation of evidence is observed that points to direct or indirect influence of bleeding as a greater determinant of subsequent adverse ischemic events. The localization (intracranial) or the intensity (gastrointestinal, retroperitoneal) of the bleeding may itself result in death. However, other consequences may exhibit harmful effects to the ACS patients or those submitted to invasive coronary procedures^[43].

CONCLUSION

The reduction of major bleeding, a relatively common complication in the current ACS scenario and possibly underestimated in randomized clinical trials, may be translated in better short- and long-term outcomes. Nowadays, its prevention represents a goal to be reached in the treatment of patients with ACS, through the balance between the risks and benefits of the pharmacological and invasive strategies offered. Appropriate risk stratification allows properly select those patients at increased risk of bleeding, focusing on them the efforts to reduce bleeding complications.

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WJC 6th Anniversary Special Issues (3): Cardiomyopathy

Infant with cardiomyopathy: When to suspect inborn errors of metabolism?

Stephanie L Byers, Can Ficicioglu

Stephanie L Byers, Can Ficicioglu, The Children's Hospital of Philadelphia, Section of Metabolic Disease, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, United States

Author contributions: Byers SL contributed to the literature review, writing paper; Ficicioglu C contributed to the literature review, designing the major elements of the paper, writing and editing the paper

Correspondence to: Can Ficicioglu, MD, PhD, The Children's Hospital of Philadelphia, Section of Metabolic Disease, Perelman School of Medicine at the University of Pennsylvania, 3501 Civic Center blvd #9054, Philadelphia, PA 19104, United States. ficicioglu@email.chop.edu

Telephone: +1-215-5903376 Fax: +1-215-5904297

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Core tip: We highlight some very helpful red flags that, when present, should point physicians in the direction of doing a metabolic workup in patients with cardiomyopathy. Short case presentations will help readers to efficiently transfer metabolic diagnostic tools in their own practice. This article will be an essential reference for physicians as they evaluate patients with cardiomyopathy.

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Abstract

Inborn errors of metabolism are identified in 5%-26% of infants and children with cardiomyopathy. Although fatty acid oxidation disorders, lysosomal and glycogen storage disorders and organic acidurias are well-known to be associated with cardiomyopathies, emerging reports suggest that mitochondrial dysfunction and congenital disorders of glycosylation may also account for a proportion of cardiomyopathies. This review article clarifies when primary care physicians and cardiologists should suspect inborn errors of metabolism in a patient with cardiomyopathy, and refer the patient to a metabolic specialist for a further metabolic work up, with specific discussions of "red flags" which should prompt additional evaluation.

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Key words: Cardiomyopathy; Inherited metabolic disorders; Inborn errors of metabolism

INTRODUCTION

Cardiomyopathy is rare in children (1.13 cases annually per 100000) but it often has catastrophic consequences including heart failure and death^[1]. While the etiology of cardiomyopathy in infancy and childhood is varied, inborn errors of metabolism cause a substantial percentage of pediatric cardiomyopathies. Determining the etiology of cardiomyopathy presenting in the first year of life is critical to ensure optimal treatment and management, provide appropriate genetic counseling, and anticipate additional medical complications which may arise.

Previously, it was reported that approximately 5% of pediatric cardiomyopathies are due to an inborn error of metabolism^[2], however a more recent study found a substantially higher percentage, with 26% of hypertrophic and 16% of dilated cardiomyopathies having a metabolic etiology^[3]. A separate study found five out of 35 infants (13.5%) diagnosed in the first year of life had a metabolic etiology to their cardiomyopathy^[4]. Over 40

Table 1 Red flags for inborn errors of metabolism associated with cardiomyopathy

Disorder	Pathognomonic biochemical abnormalities	Red flags
Mitochondrial disease	Elevated plasma lactate, elevated plasma alanine, proline	Hypotonia, developmental delays/regression, other organ involvement
Barth syndrome	Urinary excretion of 3-Methylglutaconic acid	Hypoglycemia, elevated creatine kinase, liver dysfunction, metabolic decompensation with illness
VLCAD deficiency	Elevation of C14:1 acylcarnitine species	
LCHAD deficiency	Elevation of hydroxy compounds C14-OH, C16-OH, C18-OH	
Systemic primary carnitine deficiency	Very low plasma carnitine and elevated urinary carnitine extraction	
CPT2 deficiency	Elevation of C12 to C18 acylcarnitines, notably of C16 and C18:1	
GSD deficiency II (Pompe)	Decreased acid alpha-glucosidase enzyme activity	Hypotonia, enlarged tongue
MPS1 (Hurler, Hurler-Scheie, Scheie)	Elevated urine GAGs, decreased alpha-L-iduronidase enzyme activity	Dysmorphic features (coarse features), hepatomegaly, hernia, hearing loss, corneal clouding (MPS1) developmental delays/regression
MPS2 (Hunter)	Elevated urine GAGs, decreased iduronate-2-sulphatase enzyme activity	
Propionic aciduria	Urine organic acids: 3- hydroxypropionate, Methylcitrate, Tyglylglycine, Propionyl Glycine	Hypotonia, high anion gap acidosis, hyperammonemia, metabolic decompensation with illness
Malonic aciduria	Plasma acylcarnitines: Elevated C3 (propionylcarnitine)	Developmental delay/regression, hypotonia, hypoglycemia
	Plasma acylcarnitines: Elevated C3-DC (Malonyl carnitine). Urine organic acids: elevated malonic acid	
Congenital disorders of glycosylation	Abnormal carbohydrate deficient transferrin, abnormal N- and O-glycosylation profiles (qualitative and/or quantitative)	Hypotonia, developmental delays/regression hypoglycemia, liver dysfunction

VLCAD: Very long-chain acyl-CoA dehydrogenase; GAGs: Glycosaminoglycan; CPT2: Carnitine-palmitoyl transferase deficiency; LCHAD: Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; GSD II: Glycogen storage disease type 2; MPS1: Mucopolysaccharidosis type 1.

different metabolic disorders are known to cause cardiomyopathy^[2]. Most commonly, disturbances of fatty acid oxidation, organic acidurias and storage disorders are implicated; however congenital disorders of glycosylation and mitochondrial disorders have more recently been identified in infants with cardiomyopathy^[2,3,5,6].

This review article clarifies when primary care physicians and cardiologists should suspect inborn errors of metabolism in a patient with cardiomyopathy, and refer the patient to a metabolic specialist for a further metabolic work up. Short case presentations are designed to help readers efficiently transfer metabolic diagnostic tools into their clinical practice.

WHEN TO SUSPECT A METABOLIC DIAGNOSIS IN A CHILD PRESENTING WITH CARDIOMYOPATHY

Table 1 includes a summary of some of the more common metabolic disorders associated with cardiomyopathy along with pathognomonic biochemical abnormalities. Several “red flags” may be evident in the medical history and on initial physical examination. Identification of the following “red flags” should warrant a consultation with a metabolic specialist.

Medical history

A thorough medical history, including prenatal history, may give evidence of metabolic disease. Maternal history of acute fatty liver or hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome during pregnancy may indicate that the fetus was affected with a fatty acid oxidation disorder. Newborn metabolic screening result should be obtained. A normal newborn screening is re-

assuring; however, many inborn errors of metabolism (IEM) such as storage disorders, mitochondrial disorders and congenital disorders of glycosylation are not included in the newborn screening panels; they could present with cardiomyopathy. Episodes of vomiting, lethargy, hypoglycemia, and metabolic decompensation in the context of poor feeding or illness are important clues of the potential presence of IEMs. A history of multisystem involvement, delayed developmental milestones, low muscle tone, developmental regression, coarse facial features, enlarged tongue, feeding difficulties and failure to thrive, recurrent ear and/or upper respiratory infections, rhabdomyolysis, muscle pain or spasms warrant consultation with a metabolic specialist.

Cardiomyopathy with hypoglycemia: Episodes of hypoglycemia, particularly nonketotic hypoglycemia, can be a red flag that there is a disturbance of energy production. In conjunction with cardiomyopathy, disorders of fatty acid oxidation are high on the list of differential diagnoses. Although some glycogen storage disorders may also be associated with episodic hypoglycemia, the hepatic glycogenoses are not generally associated with cardiomyopathy.

Family history

Family history of other closely related individuals with cardiomyopathy of unexplained etiology warrants further genetics evaluation. As most inborn errors of metabolism are inherited in an autosomal recessive manner, affected siblings and siblings who died at a young age from uncertain etiology should raise the suspicion for a metabolic etiology. X-linked disorders and many mitochondrial disorders are often inherited from the mother, thus family history should include second and third degree relatives,

Table 2 Biochemical testing recommendations for metabolic evaluation

Tier 1
Creatine kinase
Plasma acylcarnitine profile
Urine organic acids
Plasma lactate/pyruvate
Plasma amino acids
Enzyme analysis ¹
Tier 2
Carbohydrate deficient transferrin analysis
Urine glycosaminoglycans
Lysosomal storage disease enzyme panel (large panels are available through many laboratories)
Tier 3
Specific gene sequencing

¹If there is a high suspicion for a single metabolic disease, for example Pompe disease.

particularly on the maternal side. Mitochondrial disorders may show considerable inter-individual variation, thus focus on maternal family history for other features of mitochondrial disorders, such as migraines, seizures, stroke-like episodes, developmental disabilities/regression, movement disorders, and exercise intolerance, may provide additional indication of mitochondrial dysfunction. Information regarding parental consanguinity and ethnic origins may also increase the suspicion of a metabolic etiology.

Cardiomyopathy with hypotonia: Hypotonia can be a key indicator of systemic muscle disease not limited to the heart. In an infant, hypotonia often results in the failure to meet developmental milestones on time. Hypotonia can also manifest as difficulty feeding and respiratory distress in an infant. For an infant with severe hypotonia and cardiomyopathy, Pompe disease should be excluded from the differentials. Congenital disorders of glycosylation and mitochondrial disorders may also present with cardiomyopathy and hypotonia due to an inability to produce and utilize energy in muscle. Lastly, due to the build-up of toxic waste products, organic acidurias may present in this manner.

Physical examination

Thorough examination of the patient should be performed and focused on the following: (1) Detection of hepatosplenomegaly, hypertrophic tonsils, joint contractures (indicative of lysosomal storage disorders); (2) Assessment of a neurologic function (may be abnormal in mitochondrial disorders, storage disorders, malonic aciduria); and (3) Identification of dysmorphic features such as coarsened facial features (pathognomonic for mucopolysaccharidosis).

Hearing and vision should always be included in the exam. Involvement of multiple organ systems in a child with cardiomyopathy should increase the suspicion for an IEM.

Cardiomyopathy with hepatomegaly: Hepatomegaly is a characteristic feature of storage disorders due to accumulation of waste materials in the liver. Liver biopsy may show characteristic storage materials. These waste materials may accumulate in other areas of the body, including soft tissues, joints and bones, which may be identified on physical examination. Coarse facial features in an infant with hepatomegaly should highly increase the suspicion of a storage disorder.

Laboratory studies

Confirmation of IEMs often relies on measuring the enzyme activity and/or identifying the genetic mutations responsible, but gene sequencing and copy number analysis may take weeks to months prior to having results. In an experienced laboratory, biochemical analysis can expeditiously determine whether a metabolic etiology warrants further investigation for some IEMs. In the absence of an obvious syndromic etiology, we recommend a biochemical evaluation as a standard of care for all infants with cardiomyopathy. Specifically, we recommend an acylcarnitine profile, plasma lactate/pyruvate, creatine kinase and urine organic acids that could help in the diagnosis of fatty acid oxidation defects or malonic acidemia, which can be treated. Additional laboratory studies such as urine glycosaminoglycan quantification (for lysosomal storage disorders), N and O-glycans with carbohydrate deficient transferrin analysis (for congenital disorders of glycosylation) and specific enzyme analysis (for glycogen storage disorders and lysosomal storage disorders) may need to be performed to rule out some IEMs (Table 2).

MAJOR ETIOLOGICAL CATEGORIES

The major categories of inborn errors of metabolism associated with cardiomyopathy in infants are fatty acid oxidation disorders, lysosomal storage disorders, glycogen storage disorders, mitochondrial disorders and organic acidurias.

Fatty acids are used by the body as an alternative energy source when glucose is not available. Disorders of almost every step of the beta oxidation pathway, as well as disorders of fatty acid uptake and transport, have been identified and associated with cardiomyopathy. Carnitine-acylcarnitine translocase deficiency, carnitine palmitoyl-transferase II (CPT2) deficiency, very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency, trifunctional protein deficiency and glutaric acidemia type 2 are well known to be associated with cardiomyopathy^[7]; however others such as medium-chain acyl-CoA dehydrogenase (MCAD) deficiency have also rarely been identified in infants with cardiomyopathy^[8].

Lysosomal storage disorders (LSD) are an individually rare, but collectively common group of disorders in which waste materials accumulate in the lysosome. The accumulation of these materials in various organs and

tissues throughout the body is the main mode of pathogenesis for these disorders; however the exact mechanisms are unknown. Of the lysosomal storage disorders, Hurler syndrome (mucopolysaccharidosis type I) and Hunter syndrome (mucopolysaccharidosis type II) are the most well-known to be associated with cardiomyopathy in infancy and childhood. Maroteaux-Lamy syndrome (mucopolysaccharidosis type VI) has also been reported as presenting with cardiomyopathy in the infant period^[9]. Inheritance is autosomal recessive, with the notable exception of Hunter syndrome and Fabry syndrome, which are both X-linked. LSDs are also notable in that enzyme replacement therapies (ERTs) are available for many of these disorders. ERTs halt the further accumulation of additional waste materials in the heart, but may not fully reverse the damage already done, further stressing the importance of early diagnosis.

Caused by many enzymes involved in the synthesis and breakdown of glycogen, glycogen storage disorders have primarily either hepatic or muscle involvement. Generally, muscle glycogenoses do not have symptoms of hypoglycemia. Pompe disease, a disorder which falls into both categories of lysosomal storage disorders and glycogen storage disorders, is one of the most common metabolic disorders associated with cardiomyopathy in infants. Infantile onset is associated with extreme hypotonia, failure to thrive, respiratory distress and cardiomyopathy. Although there are juvenile and adult-onset forms of Pompe disease, cardiomyopathy is not a feature of the later onset disorder. A similar disorder, Danon disease, is X-linked and affected males exhibit cardiomyopathy, intellectual disability and myopathy. ERT is available for Pompe disease, but not Danon disease at this time. Other glycogen storage disorders, which rarely present with cardiomyopathy in the infant period, include type III (debranching enzyme deficiency)^[10] and type IV (Andersen disease)^[11].

Mitochondrial disorders typically have multisystem involvement, which can include hypertrophic or dilated cardiomyopathy, as well as left ventricular non-compaction^[6]. Although mitochondrial disorders are estimated to have an incidence of 1 in 5000 births, these disorders are likely under diagnosed. Many of the well characterized mitochondrial disorders, including Leigh syndrome, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) and myoclonic epilepsy with ragged-red fibers (MERRF), are known to include cardiomyopathy^[6].

Congenital disorders of glycosylation (CDGs) are a heterogeneous group of disorders caused by enzymatic disturbances in the synthesis of glycoproteins. The spectrum of CDGs is ever expanding. Several case reports in the literature suggest that CDGs should be considered in infants with cardiomyopathy and multisystem disorders. Infants with CDG I a (phosphomannomutase 2 deficiency) have been most often reported to have hypertrophic cardiomyopathy^[12-16] and infants with dolichol kinase deficiency have been reported to have dilated cardiomyopathy^[17,18]. Case reports exist for cardiomyopa-

thy associated with other CDGs^[12,19].

Organic acidurias are the result of enzyme deficiencies characterized by the excretion of specific organic acids in the urine. Although this group is large, only a few have been associated with cardiomyopathy. Barth syndrome, characterized by urinary excretion of 3-methylglutaconic acid due to defects in the mitochondrial protein tafazzin, causes dilated cardiomyopathy in infant males, which is often severe^[20]. Propionic acidemia is the most well known; however, individuals with propionic acidemia generally do not develop cardiomyopathy in the newborn period. Cardiomyopathy has rarely been reported in infants with methylmalonic acidemia^[21].

NEWBORN SCREENING

With the advent and standardization of neonatal screening in the United States, many metabolic disorders associated with cardiomyopathy are identified within the first days of life. Fatty acid oxidation disorders, including VLCAD deficiency, LCHAD deficiency and carnitine uptake deficiency, as well as propionic acidemia are included in the disorders recommended by the American College of Medical Genetics as part of the core panel of disorders included on the newborn screen^[22]. Despite the inclusion of several inborn errors of metabolism, this should not lead to a false sense of comprehensiveness. False negatives have been reported^[23] and individuals with fatty acid oxidation disorders may have normal acylcarnitine profiles when they are not in a state of metabolic decompensation. Lysosomal storage disorders, congenital glycosylation defects, glycogen storage disorders and mitochondrial disorders are not screened. Although many states are moving towards screening for lysosomal storage disorders, it is uncertain whether there will be universal acceptance of neonatal screening for these disorders.

CASE PRESENTATIONS

The following cases represent several infants who presented in the newborn period with cardiomyopathy and a metabolic etiology was determined.

Patient 1

He is a male infant of Puerto Rican ethnicity. He presented to an emergency department in the setting of respiratory distress. Upon evaluation, the patient was found to have pneumonia. An echocardiogram was performed, which revealed dilated cardiomyopathy with severe dysfunction. The ejection fraction was estimated at 20%. The patient was transferred to our medical center for further evaluation and management of his cardiac dysfunction.

Physical examination of the patient showed an interactive male, with frontal bossing and dysmorphic features, including depressed nasal bridge, and low set, posteriorly rotated ears. He had developmental delays and had a history of failing his newborn hearing screen. Family history was significant for consanguinity, as the patient's parents are first cousins. Family history was also remarkable for a

sister who died at age 3 years 5 mo from unspecified cardiac dysfunction.

Red flags for IEM and final diagnosis: Patient 1

Red flags: (1) Family history of sibling death due to unspecified cardiac dysfunction. Further investigation revealed that she had coarse facial features and developmental delays as well; (2) Consanguinity; and (3) Coarse facial features, dysmorphic features, hearing loss, and developmental delay.

The deceased sibling had the same signs and symptoms as this patient, and the parents are consanguineous. This suggests autosomal recessive inheritance. The patient had many other clinical findings besides dilated cardiomyopathy so this was not simply an isolated cardiomyopathy. Based on coarse facial features, hearing loss, developmental delay, and cardiomyopathy, lysosomal storage disorders such as mucopolysaccharidosis were suspected first in the differential diagnosis. Leukocyte enzyme analysis showed alpha-iduronidase activity of 0 nmol substrate per hours per milligram per protein (normal 6-71.4). This was consistent with a diagnosis of Mucopolysaccharidosis type 1, or Hurler syndrome. Genetic testing confirmed this diagnosis with homozygous c.208C > T (p.Q70X) mutations in the *IDUA* gene. Urinary glycosaminoglycan quantitation showed elevation at 163.51 mg/nmol creatinine.

Following the diagnosis of mucopolysaccharidosis type 1, this patient started enzyme replacement therapy (Aldurazyme). The patient's cardiac function has stabilized at one year of age and he will continue to be followed for signs of cardiac dysfunction.

Patient 2

He is an 8-mo-old ex-full term male born to a 32-year-old G1P0 Haitian mother and Dominican father. Pregnancy was unremarkable except for hypertrophic cardiomyopathy noted on second trimester ultrasounds that was confirmed by fetal echocardiogram. He was initially asymptomatic, but echocardiogram at birth confirmed the presence of biventricular hypertrophy with increased trabeculation and decreased left ventricular function. Cardiac catheterization and endomyocardial muscle biopsy at three weeks of life revealed non-specific findings of cardiomyopathy with muscle disarray, and there was no evidence of glycogen accumulation. Additional metabolic evaluations were unremarkable, including acylcarnitine profile, urine and plasma amino acids, ammonia, cholesterol, urine and plasma carnitine and creatine kinase. Although the lactate level was normal, pyruvate was slightly low, which caused the lactate/pyruvate ratio to be elevated at 53 (normal 10-20). Pompe disease was ruled out based on normal enzyme activity.

Red flags for IEM and final diagnosis: Patient 2

Red flags: There was no clear explanation for this patient's hypertrophic cardiomyopathy. It appeared to be an isolated cardiomyopathy without significant neurologic or other organ involvement. Elevated lactate/pyruvate

ratio was a red flag for mitochondrial disorders. This warranted further mitochondrial work up.

The patient had genetic testing for mutations in genes associated with mitochondrial disorders. The patient was found to have two predicted pathogenic variants in the *SLC25A3* gene, c.599T > G (p.L200W) and c.886-898delins7 (p.G296-S300delinsQIP). Parental testing indicated that the *SLC25A3* variants were in trans.

Mutations in *SLC25A3* are associated with mitochondrial phosphate carrier deficiency. There are only two papers in the literature describing five children from two families with mutations in this gene. Of the five children reported, three died in early infancy^[24]. Two of the other children had difficult neonatal courses, but were living at age 9 and 17 as of 2011^[25]. Mitochondrial phosphate carrier deficiency is characterized by hypertrophic cardiomyopathy, skeletal myopathy and lactic acidosis.

Patient 2 was listed for a cardiac transplant and received a heart at 8 mo of age. Following the surgery, this patient was observed to have new-onset seizures. Patient 2 continues to be followed by Cardiology, Metabolism, and Neurology at 10 mo of age.

Patient 3

He was previously reported^[26] and is included with permission of the original author. A full term male of African American ethnicity presented at 5 mo of age in the setting of decreased oral intake, fatigue with feeds, cough and fever. His prenatal history was unremarkable. His first months were significant for poor head control and gross motor delays. Echocardiogram demonstrated left ventricular dilation, spongiform appearance of the left ventricular free wall and poor ventricular functioning. The ejection fraction was shortened at 21%.

Red flags for IEM and final diagnosis: Patient 3

Red flags: Hypotonia and developmental delay, in addition to cardiomyopathy, warrant additional testing to rule out an IEM. The metabolic tests such as plasma acylcarnitine profile, blood and urine carnitine levels, creatine kinase and urine organic acid analysis should be ordered as the first step tests.

Biochemical evaluation included urine organic acids [increased excretion of malonic acid (1060 mg/g creatinine) and methylmalonate (59 mg/g creatinine)], plasma acylcarnitine profile (elevated malonyl carnitine of 0.13 nmol/mL), lactate/pyruvate (normal), and creatine kinase (normal). The patient was neither acidotic nor hypoglycemic.

Malonyl-CoA decarboxylase enzyme assay showed 12% of normal activity. Retrospective analysis of the patient's newborn screening showed an elevated malonyl carnitine of 0.39 nmol/mL, which was not reported due to lack of routine screening for this compound and lack of established standards.

This patient was treated with carnitine supplementation, medium-chain triglyceride supplementation and a high-carbohydrate diet. After one year of treatment, the

patient did not have any further episodes of metabolic decompensation, but developmental delays persisted. Follow-up cardiac surveillance continued to show left ventricle dilation with a shortening fraction of 41%.

CONCLUSION

In conclusion, determining the etiology of cardiomyopathy in the infant is critical for determination of a treatment plan, accurate genetic counseling and discussion of prognosis. A significant proportion of infants with cardiomyopathy may have a metabolic etiology and some of these benefit greatly from diagnosis and follow up treatment. The efficacy of such treatments makes it important to exclude metabolic causes for all infants presenting with cardiomyopathy.

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WJC 6th Anniversary Special Issues (3): Cardiomyopathy

Importance of genetic evaluation and testing in pediatric cardiomyopathy

Muhammad Tariq, Stephanie M Ware

Muhammad Tariq, Stephanie M Ware, Department of Pediatrics and Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN 46202, United States

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Correspondence to: Stephanie M Ware, MD, PhD, Department of Pediatrics and Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, 1044 W. Walnut Street Indianapolis, IN 46202, United States. stware@iu.edu

Telephone: +1-317-2748938 Fax: +1-317-2748679

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Abstract

Pediatric cardiomyopathies are clinically heterogeneous heart muscle disorders that are responsible for significant morbidity and mortality. Phenotypes include hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, left ventricular noncompaction and arrhythmogenic right ventricular cardiomyopathy. There is substantial evidence for a genetic contribution to pediatric cardiomyopathy. To date, more than 100 genes have been implicated in cardiomyopathy, but comprehensive genetic diagnosis has been problematic because of the large number of genes, the private nature of mutations, and difficulties in interpreting novel rare variants. This review will focus on current knowledge on the genetic etiologies of pediatric cardiomyopathy and their diagnostic relevance in clinical settings. Recent developments in sequencing technologies are greatly impacting the pace of gene discovery and clinical diagnosis. Understanding the genetic basis for pediatric cardiomyopathy and establishing genotype-

phenotype correlations may help delineate the molecular and cellular events necessary to identify potential novel therapeutic targets for heart muscle dysfunction in children.

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Key words: Pediatric; Mutation; Exome sequencing; Sarcomere

Core tip: Pediatric cardiomyopathy is a clinically and genetically heterogeneous heart muscle disease with five major phenotypes: hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, left ventricular noncompaction cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy. The genetic basis of these cardiomyopathies has been identified using traditional linkage analysis and sequencing. Novel gene discovery has been increased using modern next generation sequencing technologies, however the exact mechanisms of disease development are not fully known. In this review we focus on the current genetic knowledge of cardiomyopathies and their importance in diagnostic settings.

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INTRODUCTION

Cardiomyopathy is a clinically heterogeneous disease with a strong genetic component which affects heart muscle^[1]. In the pediatric population, 40% of children progress to death or transplantation within 5 years of diagnosis^[2-5]. The overall incidence of cardiomyopathy

Table 1 List of important genes involved in cardiomyopathy

Gene	Total coding exons	Encoded protein (AA)	NCBI GenBank accession #	Chromosomal location	Major phenotype
Sarcomere					
MYH7	38	1935	NG_007884	14q11.2	HCM, RCM, DCM, LVNC
MYBPC3	33	1274	NG_007667	11p11.2	HCM, DCM
TNNI2	15	295	NG_007556	1q32.1	HCM, RCM, DCM, LVNC
TPM1	9	284	NG_007557	15q22.2	HCM, DCM
MYL3	6	195	NG_007555	3q21.31	HCM, LVNC
MYL2	7	166	NG_007554	12q24.11	HCM, LVNC
ACTC1	6	377	NG_007553	15q14	HCM, RCM, DCM, LVNC
TNNI3	6	210	NG_007866	19q13.4	RCM
MYH6	37	1939	NC_000014	14q11.2	HCM, DCM
TNNC1	6	161	NG_008963	3p21.1	HCM, DCM, RCM
Desmosome					
JUP	9	563	NG_009090	17q21.2	ARVC
DSP	24	2871	NG_008803	6p24.3	ARVC
PKP2	14	881	NG_009000	12p11.21	ARVC
DSG2	15	1118	NG_007072	18q12.1	ARVC
DSC2	16	901	NG_008208	18q12.1	ARVC
Cytoskeleton, Z-disc, etc.					
ACTN2	21	894	NG_009081	1q43	HCM, DCM
DES	9	470	NG_008043	2q35	HCM, RCM, DCM, ARVC
LDB3	13	732	NG_008876	10q23.2	HCM, DCM, LVNC
CSRP3	5	194	NG_011932	11p15.1	HCM, DCM
TCAP	2	167	NG_008892	17q12	DCM
SGCD	8	290	NG_008693	5q33.3	DCM
TTN	311	33423	NG_011618	2q31.2	DCM
DMD	79	3385	NG_012232.1		DCM
MYPN	19	1320	NM_032578.2	10q21.3	HCM, DCM, RCM
PLN	1	52	NG_009082	6q22.31	HCM, DCM, ARVC
VCL	22	1134	NG_008868	10q22.2	HCM, DCM, LVNC
CRYAB	3	175	NG_009824	11q23.1	DCM
CAV3	2	151	NG_008797	3p25.3	HCM
BAG3	4	575	NM_004281.3	10q26.11	DCM
ANKRD1	9	319	NM_014391.2	10q23.31	HCM, DCM
Syndromic					
TAZ	11	292	NG_009634	Xq28	DCM, LVNC
ALMS1	23	4169	NG_011690	2p13.1	
PTPN11	15	593	NG_007459	12q24.13	HCM
RAF1	16	648	NG_007467	3p25.2	HCM, DCM
Others					
LAMP2	9	411	NG_007995	Xq24	HCM, DCM
LMNA	12	664	NG_008692	1q22	DCM, LVNC
EMD	6	254	NG_008677	Xq28	DCM
RYR2	105	4967	NG_008799	1q43	ARVC
ABCC9	38	1549	NG_012819	12p12.1	DCM
SCN5A	27	2015	NG_008934	3p22.2	DCM
TMEM43	12	400	NG_008975	3p25.1	ARVC

in children < 18 years of age in the United States is 1.13 cases per 100000 annually^[6,7]. Cardiomyopathy in the pediatric population is diverse and may be caused by a number of different factors, including both genetic and non-genetic etiologies, posing an intense diagnostic challenge to clinicians. As a result, the majority of cases are still considered idiopathic. More than 100 genes have been identified causing cardiomyopathy related phenotypes and these genes belong to diverse molecular pathways, implicating the involvement of contractile proteins, intracellular calcium handling, and myocardial energetics as etiologies (Table 1)^[8,9]. Identification of the underlying causes of cardiomyopathy may lead to improved outcomes with disease-specific treatments. A research-based pediatric cardiomyopathy registry (PCMR) identi-

fied familial, syndromic, neuromuscular or metabolic causes in 30% of children^[10]. In the pediatric population, sarcomeric mutations, genetic syndromes, and other unique causes such as inborn errors of metabolism, mitochondrial disorders, myopathies and neuromuscular disorders all contribute (Table 1)^[11]. However, the PCMR longitudinal outcome data on more than 3500 children with cardiomyopathy demonstrated that 60%-70% of these children are still classified as “idiopathic”^[4,5,12]. Recently, Kindel *et al*^[13] reported that classifying causes of cardiomyopathy can be increased to 70% with incorporation of evaluation by a geneticist and genetic testing. Because of the inclusion of syndromic, metabolic, and neuromuscular etiologies, genetic causes of pediatric cardiomyopathy are more heterogeneous than adult-onset

cardiomyopathy but also encompass the majority of genetic causes that result in isolated cardiomyopathy in adults (*e.g.*, sarcomeric or cytoskeletal gene mutations)^[14]. In the pediatric population, the same genetic causes that result in isolated (also termed familial) cardiomyopathy in adults are prevalent, including causes of hypertrophic cardiomyopathy (HCM; > 35% yield with sarcomeric gene panel testing) or dilated cardiomyopathy (DCM; > 20% yield with current large DCM gene panels used for testing in adults). The genetic screening of these patients for known cardiomyopathy genes helps diagnostic screening of family members, family-based risk assessment, and disease-management^[13,15,16]. Historically, this immense genetic and allelic heterogeneity has made molecular analyses difficult, expensive, and time-consuming due to low throughput of traditional sequencing technologies. However, recent advances in sequencing technologies provide rapid, accurate, and cost-effective DNA sequencing. The majority of the clinical diagnostic laboratories are now adopting next generation technologies for their routine gene testing in cardiomyopathy and focusing on coding regions. It is estimated that about 85% of disease-causing mutations lie within the protein-coding regions of the human genome^[17-19].

Cardiomyopathy is classified into 5 clinical phenotypes: HCM, DCM, restrictive cardiomyopathy (RCM), left ventricular noncompaction cardiomyopathy (LVNC), and arrhythmogenic right ventricular cardiomyopathy (ARVC)^[20,21]. Although these are clinically distinct entities, there is evidence for genetic overlap among them. For example, mutations in beta myosin heavy chain (*MYH7*) are most commonly associated with HCM and DCM but have also been reported in RCM^[14,22] and LVNC^[23-25]. The majority of pediatric cardiomyopathy cases exhibit dilated (50%) or hypertrophic (42%) phenotypes^[6,26]. The PCMR is a valuable source for this population in terms of outcome and clinical features. In this review we will focus on the genetic causes of cardiomyopathy in the pediatric population.

HCM

HCM is the most prevalent inherited cardiac disorder and is defined as the presence of unexplained left ventricular hypertrophy (LVH), a primary myocardial process, with myocyte disarray and fibrosis. Fibrosis is a common endpoint in the pathological process of HCM. HCM was the first cardiomyopathy with a specific genetic etiology identified^[27,28]. HCM is also considered the most common cause of sudden cardiac death in young, healthy and athletic individuals^[29]. In adults, the diagnosis of HCM implies a sarcomeric gene mutation as the underlying etiology. However, in children, HCM is a heterogeneous group of disorders encompassing conditions with diverse genetic origins and clinical phenotypes, including associations with inborn errors of metabolism, neuromuscular disorders, and malformation syndromes^[6,10,13,30,31]. This is an important clinical distinction since patients classified

in the metabolic, syndromic, or neuromuscular categories have additional medical management needs. At times, these conditions may require a high level of clinical suspicion in order to diagnose at early ages. For example, at our institution, the incorporation of genetic evaluation into the cardiomyopathy population led to the diagnosis of Noonan syndrome or Noonan syndrome with multiple lentigines in several adolescents and young adults who had been followed since early childhood with presumed isolated sarcomeric HCM and who had only very subtle features of a syndromic cause. These diagnoses also have substantial implications for family based cardiac screening recommendations.

HCM is frequently inherited in an autosomal dominant manner with hundreds of mutations affecting more than 27 genes identified to date (Table 1). Over 1000 distinct mutations in sarcomeric genes (*MYBPC3*, *MYH7*, *TNNT2*, *TPM1*, *ACTC1*, *TNNI3*, *TTN*, *MYL2*) of the contractile apparatus are known to cause adult-onset HCM^[32,33] leading to the paradigm that HCM is a disease of the sarcomere^[34,35]. Mutations in *MYH7*, encoding beta-myosin heavy chain, and in *MYBPC3*, encoding cardiac myosin-binding protein C, are the most common, each accounting for approximately 40% of all cases and nearly 80% of all mutation positive cases; the remaining seven genes each account for less than 1% to 5% of cases and collectively 10% of cases^[36]. Overall, pathogenic mutations have been identified in 50%-70% of HCM cases^[37]. Mutations found in these genes are generally missense, incorporating a mutated protein into the sarcomere. An exception is the *MYBPC3* gene, in which half of the mutations are truncations causing haploinsufficiency of the protein^[38,39]. Interestingly, in the pediatric population, *MYBPC3* truncating mutations are less common and missense mutations predominate. Until recently, mutations in the sarcomeric machinery were thought to cause HCM in adults only and not contribute significantly to the development of HCM in young children^[40]. However, two independent reports have shown that as many as 50% of pediatric HCM cases harbor mutations in sarcomeric genes and 17% of patients with these sarcomeric mutations were diagnosed in the first year of life^[14,41], suggesting that sarcomere gene mutations are important cause of HCM both in adults and pediatric populations. Following this, Kindel *et al*^[13] reported sarcomeric gene mutations as the major cause of disease in pediatric HCM patients with a family history of the disease. Non-genetic causes rarely cause HCM in children although LVH can occur in response to some environmental triggers, such as transient LVH in infants of diabetic mothers^[42]. Both RCM and HCM are characterized by diastolic dysfunction and some reports suggest a clinical overlap with distinct clinical outcomes for patients who exhibit HCM with restrictive physiology^[43,44]. In some families, distinct HCM and RCM phenotypes segregate with the same disease causing sarcomeric mutation^[45]. Recently, risk factors for the outcomes of death or transplantation were reported for the largest pediatric HCM cohort studied to date^[26]. The

results demonstrated that risk was greatest for those who presented as infants, those with inborn errors of metabolism, or those with mixed HCM phenotypes (HCM and DCM or HCM with restrictive physiology). Interestingly, children with mixed HCM with DCM or RCM phenotype frequently have a family history of the disease including family members with isolated HCM or mixed phenotypes^[26], suggesting that even in families with Mendelian inheritance of cardiomyopathy, more complex genetic interactions occur to determine phenotype, with genetic modifier factors involved.

In the pediatric population, if metabolic or syndromic causes are ruled out as etiologies, HCM is considered a familial disease caused by the same genes that are causal for isolated cardiomyopathy in adults. The diagnosis of HCM in a child with suspected isolated cardiomyopathy should prompt evaluation of the first-degree relatives^[46,47]. Current guidelines indicate that cascade cardiac screening and genetic testing are indicated in this patient population. These cascade screening and testing approaches have been applied particularly successfully in the Netherlands, where a founder *MYBPC3* mutation results in an identifiable at risk population^[48]. Miller *et al*^[49], assessed the success of cascade cardiac screening and genetic testing in a pediatric population in the United States, the first study to examine this approach in the United States. Cardiac screening of at-risk relatives in HCM families identified disease in a subset of asymptomatic relatives (25%). Interestingly, the study found that the uptake of cardiac screening was significantly higher than the uptake of genetic testing. The reasons for this are unclear given that known familial mutation genetic testing is substantially less expensive than an echocardiogram in the United States and also takes less time for the actual procedure (blood draw as compared to echocardiogram). Additional studies are important to determine the best delivery methods of cost effective familial screening and appropriate genetic testing.

RCM

RCM is a rare and distinct form of cardiomyopathy characterized by diastolic dysfunction but intact systolic function until later stages of the disease. The main features are marked atrial enlargement, and normal ventricular wall thickness (no hypertrophy)^[50]. It accounts for less than 5% of all cardiomyopathies in the United States and Europe^[51,52]. RCM is also an uncommon cardiomyopathy in children, accounting for approximately 3%-5% of all cardiomyopathy cases. Among the different types of cardiomyopathies, RCM has the worst prognosis, especially in pediatric cases where heart transplantation is often the only effective treatment^[44,52,53]. To date, dominant mutations causing pediatric RCM have been reported with *DES*, *ACTC1*, *TNNI3*, *TNNT2*, and *MYH7* genes, but the majority of cases are considered idiopathic^[8,22,54]. Recently, a *de novo* mutation in titin (*TTN*) was reported causing familial RCM^[55]. Webber *et al*^[52] described the

largest RCM cohort ($n = 152$; 4.5% of all pediatric cardiomyopathy cases within the PCMR cohort) with one-fourth with a family history of the disease, indicating a genetic contribution to the disease, and one-third ($n = 51$) with a mixed/overlapping phenotype of RCM/HCM, suggesting that additional shared genetic causes may exist. One of the interesting questions for future research will be to understand how mutations in the same gene can cause distinct phenotypes. For example, mutations in *MYH7* can cause HCM, RCM, DCM, or LVNC. Possible explanations include mutation location resulting in protein domain specific phenotypic effects or effects of genetic modifiers. Future research will further delineate the consequences of specific mutations by highlighting the effects on protein-protein interactions and more precisely delineating specific patterns of genetic network dysregulation in response to mutational change.

DCM

DCM is characterized by left ventricular dilation and systolic dysfunction. The estimated annual incidence of DCM in children is 0.57 cases per 100000, with overall poor prognosis, and with 40% of children undergoing cardiac transplant or dying before 5 years post-diagnosis^[4,6,10,56,57]. Pediatric DCM is the commonest form of cardiomyopathy, accounting for approximately 60% of all cases^[58]. While environmental causes (predominantly related to infections resulting in myocarditis) contribute substantially to DCM in the pediatric population, a significant family history of DCM is not uncommon in pediatric patients, and the same genes that cause DCM in adults have been shown to lead to earlier onset DCM as well^[59,60]. DCM is the most genetically heterogeneous of all cardiomyopathies with all Mendelian patterns of inheritance represented (autosomal dominant, autosomal recessive, X-linked, and mitochondrial)^[61,62]. Neuromuscular causes of DCM, such as Duchenne muscular dystrophy, are relatively common in the pediatric population. In addition, inborn errors of metabolism and mitochondrial disorders underlie up to 10%-15% of cases in the pediatric population^[13]. Syndromic causes of DCM are rare but do occur and are likely under-recognized^[63]. Genetic causes of familial DCM are identified in approximately 30% of cases. To date, more than 40 genes have been identified for non-syndromic forms of DCM in adults, though only 3 of them (*TNNI3*, *GATAD1* and *DOLK*) show autosomal recessive inheritance^[64-66]. Genetic causes of autosomal recessive forms of DCM have rarely been identified, although they are thought to explain approximately 16% of familial DCM and contribute to sudden cardiac death and heart failure, especially in the pediatric population. DCM is predominantly caused by mutations in genes encoding cytoskeletal and sarcomeric proteins^[67-69]. Recently, heterozygous truncating mutations in *TTN* were reported in 25% of DCM cases, suggesting that the diagnostic yield for DCM might increase substantially with the addition of *TTN* sequenc-

ing to current gene testing panels^[70,71]. However, truncating *TTN* mutations have been also reported in 3% of a healthy control populations^[70], raising the possibility of a complex genetic model for DCM and posing a problem for clinical interpretation of many *TTN* variants. The prevalence of mutations in *TTN* has not been reported in children with DCM, although clearly there are shared genetic causes. Identification of the genetic causation of DCM allows for appropriate surveillance in neonates, infants, and children with DCM.

The Heart Failure Society of America has published recommended guidelines for genetic evaluation of DCM including family history, periodic cardiovascular screening of at-risk family members, and consideration of genetic counseling for DCM patients, and, when applicable, their family members. Upon targeted gene testing, unaffected family members with positive genetic testing results should undergo cardiac screening once a year. If mutation testing in the proband is negative or not performed, first degree relatives should undergo cardiac screening every 3-5 years^[62]. Gene panels for DCM are quite large with > 50 genes available. However, these panels do not typically include the most common neuromuscular, syndromic, and metabolic causes of DCM in childhood, making it important to identify a differential with regard to cause and perform the correct testing to address suspected cause. This requires an understanding of the most common causes of DCM, careful attention to phenotyping beyond the cardiac condition, and knowledge of different types of genetic testing in order to facilitate the most appropriate and/or tiered testing as applicable.

ARVC

ARVC is characterized by a high incidence of ventricular arrhythmia and sudden death with an estimated prevalence of 1:2000 to 1:5000 in the general population^[72,73]. ARVC is an inherited disorder with a family history in 30% to 50% of the cases (Klauke, 2010). ARVC is predominately reported as autosomal dominant trait, though autosomal recessive cases have been observed, frequently with syndromic features including cutaneous findings. ARVC has been considered a desmosomal disease caused by mutations in five desmosomal genes (*PKP2*, *DSP*, *JUP*, *DSG2*, *DSC2*) in approximately 50% of total cases, however other non-desmosomal genes are known to be responsible for the disease (*TMEM43*, *PLN*, *RYR2*, *LMNA*, *TTN*, *CTNNA3*, *TBF-β3*)^[74-80]. ARVC is not frequently found in the pediatric population, however a recent Danish nationwide study reported sudden cardiac death in children ($n = 4$) due to ARVC^[81].

LVNC

LVNC is a distinct rare form of primary cardiomyopathy with a genetic origin which is characterized by excessive trabeculation of the left ventricular myocardium, progressive myocardial dysfunction, and early mortality. Clinical

presentation includes arrhythmia and sudden cardiac death. Current studies in children estimate that LVNC accounts for approximately 9% of newly diagnosed cardiomyopathies^[58,82]. Recently, Brescia *et al.*^[83] retrospectively reported a cohort of pediatric LVNC ($n = 242$) with a high mortality rate and a strong association with arrhythmias. Criteria for “excessive” trabeculation have been proposed, but the diagnosis of LVNC is often more controversial than other cardiomyopathy phenotypes. In addition, LVNC may present as a mixed cardiomyopathy seen in combination with DCM or HCM, or may present in conjunction with congenital heart defects^[84].

LVNC is a genetically heterogeneous disease that may be inherited in an X-linked, recessive, or autosomal dominant pattern. To date, genetic causes of LVNC have been implicated in genes encoding sarcomeric, cytoskeletal, sodium channel and unknown function proteins, *i.e.*, tafazzin, *DTNA*, *LDB3*, *ACTC1*, *MYH7*, *TNNT2*, and *SCN5A*^[84]. The identification of LVNC in patients with mitochondrial disorders is not uncommon, as was initially seen for patients with Barth syndrome, caused by mutations in tafazzin. Mitochondrial genome mutations have also been revealed in patients with isolated LVNC as evident by biopsies from patients with mitochondrial abnormalities^[85]. These causes of LVNC are rare in the general population and the genetic basis of disease remains unknown in a large proportion of patients. We screened 31 cardiomyopathy genes (sarcomeric and non-sarcomeric) in 23 childhood isolated LVNC patients using a custom next generation sequencing platform. This identified 13 previously known and 10 novel disease-causing mutations in 18 patients, predominantly in the *MYBPC3* gene (unpublished results). Further extensive genetic analyses will unravel novel and previously associated with other types of cardiomyopathy cause for LVNC, supporting the hypothesis of shared genetic etiology of cardiomyopathies.

CLINICAL GENETIC TESTING IN CARDIOMYOPATHY

Progress in understanding the genetic basis of cardiomyopathy enhances the value of clinical genetic testing and provides the clinician an additional route to diagnose individuals at risk for cardiomyopathy and understand pathogenesis. Newer technologies are influencing cardiomyopathy genetic testing, where an increased number of genes are now routinely being tested simultaneously, and enhancing the diagnostic yield and utility. However, simple statistics dictate that the more genes that are tested, the more variants of uncertain significance (VUS) will be discovered. VUS results can present a clinical challenge for care providers not comfortable with genetic testing results and can also present challenges for discussion and interpretation for families. Targeted next-generation based sequencing for cardiomyopathy gene panels are available through various laboratories in the United States and worldwide (<http://www.genetests.org>)

and <http://www.ncbi.nlm.nih.gov/gtr>). Genetic testing in HCM has the highest diagnostic yield and therefore clinical utility^[86]. The yield of current testing is approximately 60% for familial and approximately 40% for sporadic HCM cases^[36]. The Heart Rhythm Society and European Heart Rhythm Association guidelines recommended the comprehensive screening of 5 sarcomere genes (*MYBPC3*, *MYH7*, *TPM1*, *TNNI3*, *TNNT2*) for HCM^[87], although these recommendations pre-date the rapid expansion in the number of genes tested on current clinical gene panels. Currently, genotype-phenotype correlations in HCM are controversial although there is a general consensus that incorporation of the genetic testing results should be part of management discussions. The sophistication to provide a specific prognosis based on, for example, a mutation in the N-terminal vs C-terminal domain of *MYH7* is not currently present. However, genotype-phenotype correlations exist for certain genes. For example, mutations in *LMNA* may result in a number of extra-cardiac features that require surveillance and management, but patients with these mutations may present with isolated DCM. Genetic testing of HCM is particularly useful for screening potential at risk first-degree relatives and subsequent cascade testing of family members as indicated. In a recent Danish study, child relatives (< 18 years of age) of HCM families were assessed based on clinical and predictive genetic testing and 6% of the asymptomatic relatives at-risk of HCM were found to develop HCM after a 12-year follow-up^[16]. Hofman *et al*^[15] assessed the yield of genetic testing in 648 HCM families from the Netherlands and found a 46% yield for positive genetic testing in probands with cascade screening of mutation positive families revealing 489 mutation-positive subjects over a 15-year follow-up. In DCM, the mutation spectrum is broader and detection rates are less than HCM owing to higher locus and allelic heterogeneity. However recent novel gene discoveries (for example *BAG3*, *RBM20*) are resulting in continuous additions to DCM gene panels. Also, the recent discovery of the high contribution of *TTN* mutations (25% familial and 18% sporadic) to DCM may increase the mutation detection rates in genetic testing panels to closer to that of HCM although the rates of *TTN* mutations segregating with disease need to be validated in larger populations^[70].

CHALLENGES INTO THE GENETICS OF PEDIATRIC CARDIOMYOPATHY

Despite the advancements in genetic and genomic technologies, multiple challenges remain in order to clearly delineate the complete genetic etiologies responsible for pediatric cardiomyopathy. Pediatric cardiomyopathy is a very heterogeneous entity with variable phenotypes are seen within and between families even with identical genetic causes. Another complicating factor is the complex genetics of the disease. Although the majority of known isolated cardiomyopathy cases are caused by single gene mutations, it is important to remember that variants in

more than one gene may be involved in disease causation. Identifying genetic modifiers is the next important step in pediatric cardiomyopathy genetic research and may be important to identify the causes of phenotypic variability within members of the same family. The high cost of traditional sequencing technologies posed a severe limitation to the discovery of new disease genes and screening of known disease genes in the past. New technology circumvents this hurdle, but the current challenge is to provide accurate and clinically useful interpretation of the variants identified in order to maximize the clinical utility of testing. Of course, the reproducibility of the next generation sequencing such as exome sequencing, is very high, however we do not have a complete expertise to identify the causative culprits from thousands of genetic variants. Differentiation of pathogenic variants, disease modifiers, and rare, benign variants in the deluge of data emerging from increasingly accessible novel sequencing technologies (> 80 K variants per exome and approximately 3 million per whole genome) is a challenge. This requires another tier of extensive research to understand the nature of disease causing variants available from advanced high-throughput sequencers. In this context, the involvement of pediatric cardiologists is very important in order to provide careful and comprehensive phenotypic information before genetic testing and/or evaluation. Finally, delineating the complex interplay of genes and environment and their relative contribution to phenotypic presentation and disease course is important for management and prognosis.

CONCLUSION

Modern genomics and human genetics have the capability to decipher the complete genetic anatomy of heritable pediatric cardiomyopathy. Early diagnosis and identification of at risk individuals is important as the clinical implications and outcomes may vary depending on both the gene and mutation type. While next-generation sequencing technologies have increased the capacity of genetic testing by an order of magnitude, we need extensive phenotyping expertise in order to inform novel gene discovery and interpretation of identified variants. In addition, genetic counseling of affected families is critical to facilitate testing and ensure appropriate pre- and post-test understanding of testing implications and results. Identification of the genetic modifiers is an important step toward a personalized medicine approach, but will require analysis of large cohorts using newer sequence capture technologies. Identification of the molecular etiology will allow sub-classification of pediatric cardiomyopathy based on cause. Understanding rare variants and SNPs that modify disease presentation and progression hold the promise of allowing new therapies to be developed.

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WJC 6th Anniversary Special Issues (3): Cardiomyopathy

Diagnosis and management of ischemic cardiomyopathy: Role of cardiovascular magnetic resonance imaging

Christina Doesch, Theano Papavassiliu

Christina Doesch, Theano Papavassiliu, 1st Department of Medicine Cardiology, University Medical Center Mannheim, Medical Faculty Mannheim, University of Heidelberg, 68167 Mannheim, Germany

Christina Doesch, Theano Papavassiliu, DZHK (German Centre for Cardiovascular Research) partner site Mannheim, 69117 Heidelberg, Germany

Author contributions: Doesch C reviewed the literature, organized and wrote the manuscript; Papavassiliu T reviewed the literature, critically reviewed and edited the final version of the manuscript.

Correspondence to: Theano Papavassiliu, MD, 1st Department of Medicine Cardiology, University Medical Center Mannheim, Medical Faculty Mannheim, University of Heidelberg, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany. theano.papavassiliu@umm.de

Telephone: +49-621-3832204 Fax: +49-621-3833821

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Abstract

Coronary artery disease (CAD) represents an important cause of mortality. Cardiovascular magnetic resonance (CMR) imaging evolved as an imaging modality that allows the assessment of myocardial function, perfusion, contractile reserve and extent of fibrosis in a single comprehensive exam. This review highlights the role of CMR in the differential diagnosis of acute chest pain by detecting the location of obstructive CAD or necrosis and identifying other conditions like stress cardiomyopathy or myocarditis that can present with acute chest pain. Besides, it underlines the prognostic implication of perfusion abnormalities in the setting of acute chest pain. Furthermore, the review addresses the role of CMR to detect significant CAD in patients with stable CAD. It elucidates the accuracy and clinical utility of CMR with respect to other imaging modalities

like single-photon emission computed tomography and positron emission tomography. Besides, the prognostic value of CMR stress testing is discussed. Additionally, it summarizes the available CMR techniques to assess myocardial viability and describes algorithm to identify those patient who might profit from revascularization those who should be treated medically. Finally, future promising imaging techniques that will provide further insights into the fundamental disease processes in ischemic cardiomyopathy are discussed.

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Key words: Coronary artery disease; Cardiovascular magnetic resonance imaging; Prognostic value; Stress testing; Viability

Core tip: Coronary artery disease (CAD) represents an important cause of mortality. This review highlights the role of cardiovascular magnetic resonance (CMR) in the differential diagnosis of acute chest pain. It underlines the prognostic implication of perfusion abnormalities in the setting of acute chest pain and addresses the role of CMR to detect significant CAD in patients with stable CAD. Besides, the prognostic value of CMR stress testing is discussed. Additionally, it summarizes the available CMR techniques to assess myocardial viability. This review describes a treatment algorithm and presents new imaging techniques that might give further insights into the fundamental disease processes in ischemic cardiomyopathy.

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INTRODUCTION

Coronary artery disease (CAD) has a high prevalence in industrialized countries^[1] and is therefore an important cause of mortality in the Western world^[2]. Cardiac magnetic resonance (CMR) imaging offers the unique opportunity to non-invasively detect coronary artery stenoses and has become the gold standard for the assessment of viability. The detection of coronary artery stenoses can be performed using either vasodilator stressors like adenosine to detect myocardial ischemia or inotropic agents such as dobutamine to identify regional wall motion abnormalities. Due to its excellent temporal and spatial resolution, the possibility to assess myocardial perfusion without exposure to ionizing radiation and the independence of an acoustic window, CMR offers plenty advantages over other imaging modalities like stress echocardiography or single-photon emission computed tomography (SPECT).

CMR TESTING IN PATIENTS WITH ACUTE CHEST PAIN

The exclusion of coronary artery stenoses in patients presenting with acute chest pain in the absence of diagnostic electrocardiographic changes or negative cardiac enzymes still remains a challenge. In these low risk patients CMR has proved to be a reliable risk-stratification tool. Kwong *et al.*^[3] was the first to demonstrate the utility of CMR for triage of patients with acute chest pain in the emergency department. He showed that the combination of CMR rest perfusion and late gadolinium enhancement (LGE) in patients presenting at an emergency department with angina and non-diagnostic electrocardiogram (ECG) had a sensitivity of 100% for non-ST-segment elevation infarction and a sensitivity of 84% sensitivity for acute coronary syndrome (ACS) as well as a specificity of 85% (Figure 1). Besides, CMR proved to be the strongest predictor of ACS and had an independent diagnostic value over clinical parameters including ECG, initial troponin-I, and the thrombolysis in myocardial infarction risk score. In a further study by Ingkanisorn *et al.*^[4], adenosine stress CMR was performed in 135 patients with chest pain and excluded myocardial infarction who presented at the emergency department. In this setting, adenosine perfusion abnormalities had 100% sensitivity and 93% to predict CAD. Furthermore, none of the patients with a normal adenosine stress examination was diagnosed with significant CAD or suffered from an adverse outcome during a follow-up period of one year. In a retrospective study by Hartlage *et al.*^[5] using either adenosine or dobutamine stress CMR in 255 patients presenting at the emergency department with acute low-risk chest pain and no prior history of CAD the negative predictive value for the primary endpoint of cardiac death, nonfatal acute myocardial infarction, obstructive CAD on invasive coronary angiography or recurrent chest pain, was 100% and 99%, respectively. Therefore, adenosine and dobutamine

stress CMR proved to be reliable modalities to exclude obstructive CAD and a negative stress study provides an excellent intermediate-term prognosis. Besides, in patients with intermediate risk presenting at the emergency department, stress CMR reduced cardiac-related costs of the index visit and over the first year without increasing major cardiac events^[6].

In addition, CMR can identify the underlying cause of conditions that present like ACS. In stress cardiomyopathy [Takotsubo cardiomyopathy (CMP), Figure 2], patients present with acute chest pain and/or dyspnea, modest elevation in cardiac troponin level and new ECG abnormalities despite the absence of significant (> 50%) obstructive coronary artery disease or angiographic evidence of acute plaque rupture. In these patients with marked apical or midventricular ballooning the absence of myocarditis or typical ischemic transmural LGE on CMR confirms the diagnosis^[7-11]. Myocarditis (Figure 3) is another differential diagnosis in patients with acute chest pain that can be addressed with CMR allowing to visualize the key features of myocarditis: inflammation, hyperemia, edema, necrosis, myocardial dysfunction as well as accompanying pericardial perfusion in a single study^[12-16].

CMR STRESS TESTING IN PATIENTS WITH STABLE CAD

The feasibility of stress CMR to detect coronary artery stenosis in patients with known or suspected CAD is well established^[17-21]. In a meta-analysis^[22] comparing 114 SPECT, 15 positron emission tomography (PET) and 37 CMR myocardial perfusion imaging studies for the detection of angiographically detected coronary artery stenoses $\geq 50\%$, all three imaging modalities proved to accurately detect obstructive CAD. Metaregression showed that CMR and PET have a significantly higher diagnostic accuracy than SPECT. In contrast to nuclear techniques, CMR perfusion is not affected by attenuation artifacts, has the highest spatial resolution and is therefore able to even subendocardial perfusion deficits^[23]. The sensitivity and specificity to detect CAD ranged between 79%-88% and 81%-91% for dobutamine stress CMR or 67%-94% and 61%-85% for adenosine stress CMR, in meta-analysis^[24-26] and a multicenter study^[27]. The use of 3.0 T has shown to provide even higher diagnostic accuracy^[28,29], however this technique is not widely available, yet and no data from multicenter studies exist so far.

However, CMR stress testing is not only able to detect CAD but also offers prognostic information. A study performing adenosine stress CMR using 1.5 and 3.0 T in 815 consecutive patients with stable CAD could show that the addition of inducible ischemia reclassified patient risk beyond standard clinical variables and improved discrimination of major adverse cardiac events^[30]. These results were confirmed by another single center study^[31] enrolling 1229 patients with stable angina. Recent meta-analysis^[32,33] also proved that a negative adenosine or dobutamine stress CMR had a high negative predictive

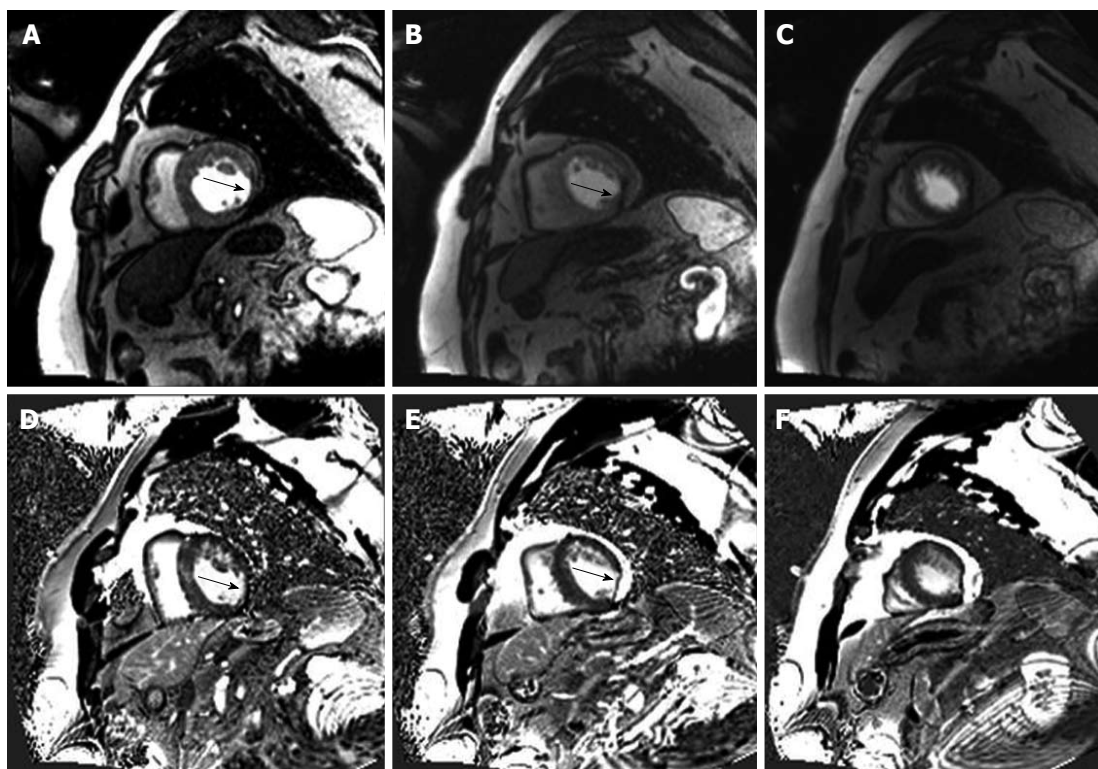


Figure 1 Patient presenting with an subacute non-ST-segment elevation infarction. Cardiovascular magnetic resonance (CMR) images of a 54-year-old man who presented with typical chest pain. Troponin was elevated to 1.9 $\mu\text{g/L}$. CMR rest perfusion (A-C) shows a subendocardial perfusion deficit inferolateral and lateral on the basal (A) and midventricular (B) short axis slice. The black arrow highlights the subendocardial perfusion deficit. Late gadolinium enhancement (D-F) of the representative short axis also revealed a hyperenhancement inferolateral and lateral (black arrow) indicative of a subacute myocardial infarction.

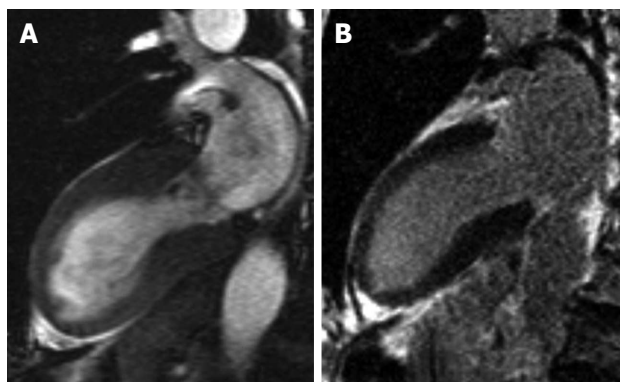


Figure 2 Patient with takotsubo cardiomyopathy. Example of a 45-year-old woman presenting with acute chest pain, anterior ST-segment elevation on electrocardiogram. Cardiovascular magnetic resonance cine images showed a typical apical ballooning of the left ventricle (A). Late gadolinium enhancement images (B) could rule out myocardial infarction and did not show any fibrosis.

value for adverse cardiac events. Besides they showed that inducible perfusion defects as well as wall motion abnormalities had a comparable ability to identify low-risk patients.

Therefore, in the actual guidelines for the management of patients with stable CAD, stress imaging using either echocardiography, CMR or SPECT has become an integral part in the work-up of patients with a pretest probability (PTP) of CAD between 15%-65% and a left ventricular function (LVEF) $\geq 50\%$ as well as in patients

with a PTP of 66%-85% or a LVEF $< 50\%$ ^[34]. An imaging study should also be considered in symptomatic patients with prior revascularization [percutaneous coronary intervention (PCI) or coronary artery bypass graft]^[34]. In patients with coronary artery stenoses of angiographic intermediate severity causing a perfusion defect on CMR it could be shown that these patients are at higher risk for major adverse cardiac events (MACE) within the following 18 mo after the procedure, whereas deferring PCI in patients with intermediate coronary artery stenoses and no evidence of ischemia seemed to be safe^[18]. Thus, current guidelines suggest to consider an imaging stress test to assess the functional severity of intermediate lesions on coronary arteriography^[34]. The decision to proceed to invasive angiography is not only based on symptoms and risk factors but also on the extent and severity of ischemia^[34] (Figure 4).

GENDER-BASED PROGNOSTIC VALUE OF CMR STRESS TESTING

In women, CAD develops 7 to 10 years later than in men. However, it is still the major cause of death in women^[35]. Moreover, the risk of heart disease in women is often underestimated. Due to the underrecognition of heart disease and differences in clinical presentation in women, treatment strategies are less straightforward in women. In a study by Coelho-Filho *et al*^[36] performing adenosine

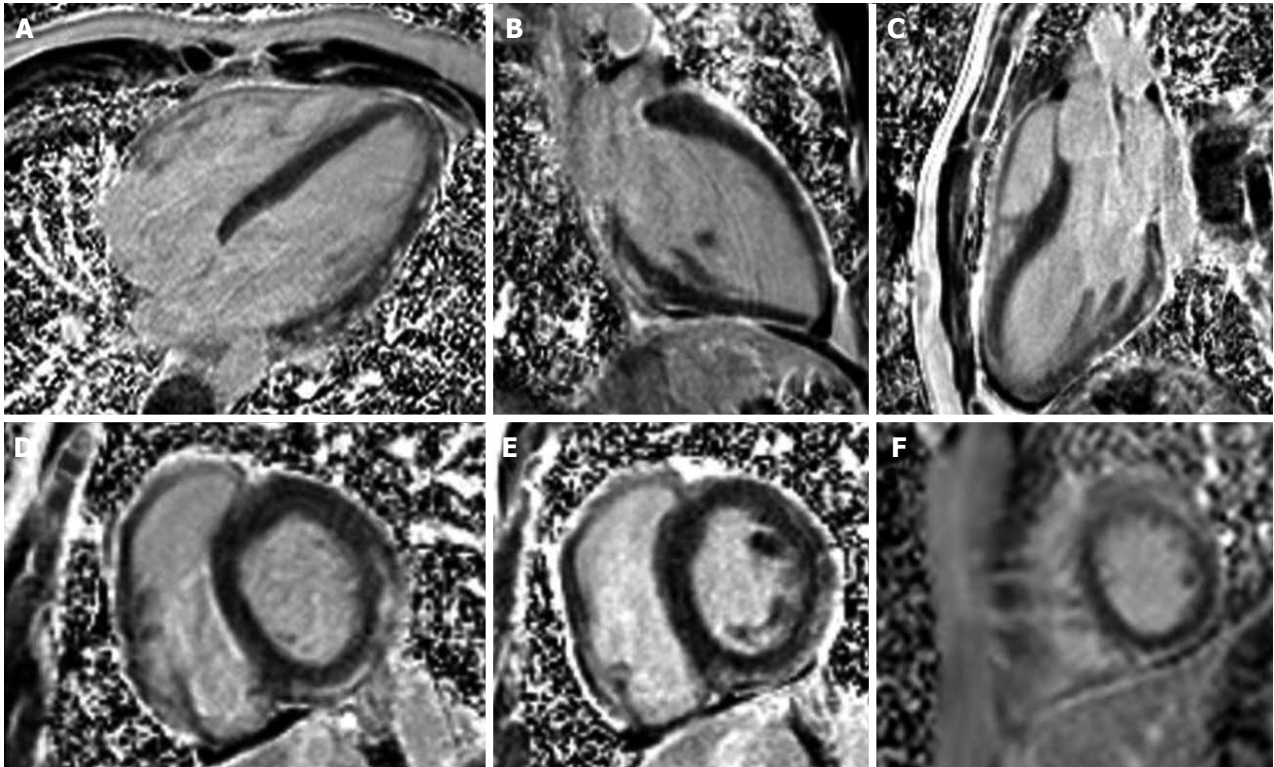


Figure 3 Patient with acute chest pain due to myocarditis. A 16-year-old boy who presented with acute chest pain and palpitations 1 wk after a gastrointestinal infection. Troponin was 2.4 $\mu\text{g/L}$. Late gadolinium enhancement cardiovascular magnetic resonance showed a patchy midmyocardial and epicardial hyperenhancement of the lateral, anterior and inferior wall. These findings are typical of acute myocarditis.

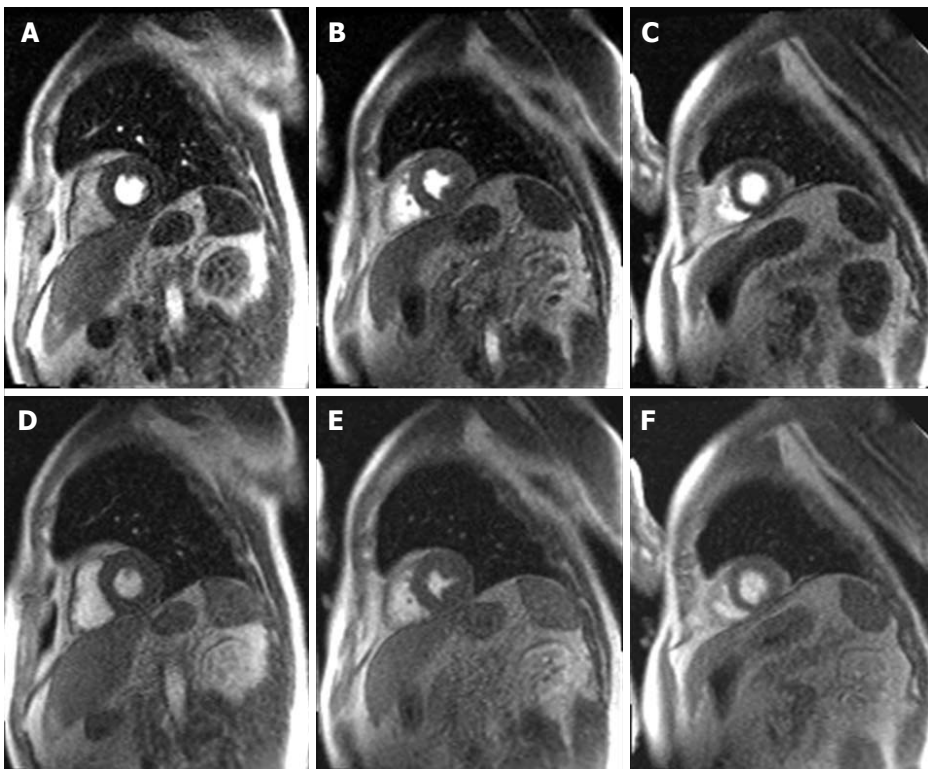


Figure 4 Adenosine stress perfusion imaging. A 63-year-old patient who presented with stable angina for more than 6 mo. Adenosine stress (A-C) vs rest perfusion (D-F) revealed myocardial ischemia only during stress perfusion of the basal inferior and lateral as well as midventricular inferoseptal wall. The patient did not show a late gadolinium enhancement. Coronary angiography showed a 60% stenosis of the medial right coronary artery that was treated with percutaneous coronary intervention and stent implantation due to the detected ischemia.

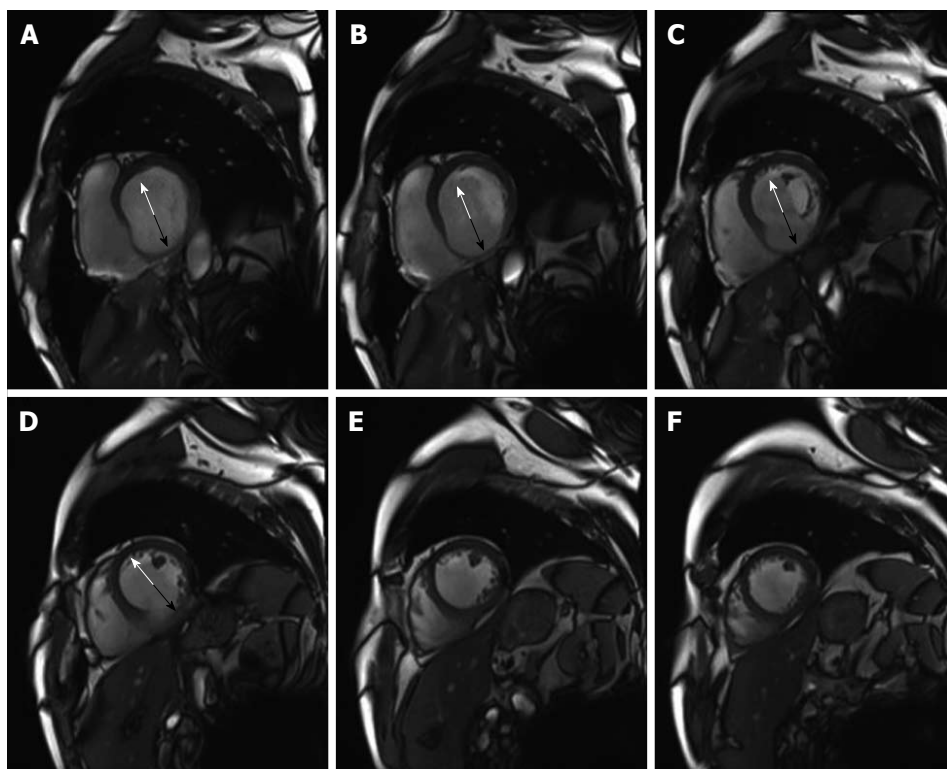


Figure 5 End diastolic wall thickness. Representative end diastolic short axis images from basal (A) to apical (F) of a patient with previous inferior myocardial infarction. The anterior, septal and lateral region (white arrow, A-D) show a preserved end diastolic wall thickness (EDWT) > 6 mm suggesting viable myocardium, whereas EDWT of the inferior wall (black arrow, A-D) is \leq 6 mm indicating myocardial scarring.

stress imaging in 237 men and 168 women referred for ischemia assessment, myocardial ischemia was the strongest predictor of MACE in both sexes. In a large study^[37] using a combined adenosine and dobutamine stress CMR protocol in 471 men and 208 women, Jahnke *et al.*^[37] could show that CMR perfusion and wall motion abnormalities are equally suited for cardiac risk stratification in both sexes. In women, a negative stress CMR resulted in very low event rates during the following 4 years whereas, the event rates in men increased after the second year. These results might suggest that it is feasible to prolong the generally proposed 2-year warranty period of a negative CMR stress test to 4 years in women.

ROLE OF CMR IN THE DETECTION OF MYOCARDIAL VIABILITY IN ISCHEMIC HEART DISEASE

In clinical practice myocardial viability is characterized by functional recovery 6 wk to 6 mo after successful revascularization. CMR offers 3 methods to assess myocardial viability: end-diastolic wall thickness, low dose dobutamine stress CMR and LGE.

The easiest technique is to evaluate the maximal end-diastolic wall thickness (EDWT) because it only requires to determine the maximal EDWT on the cine images at rest. In the course of acute myocardial infarction structural changes are associated with myocardial thinning in

the core zone of the infarction. In a study comparing EDWT on resting CMR and^[18] fluorodeoxyglucose positron emission tomography (FDG PET) in 35 patients with myocardial infarction, Baer *et al.*^[38] could prove that myocardial segments with an EDWT \geq 5.5 mm showed a normal FDG uptake, whereas myocardial segments with an EDWT < 5.5 mm revealed a significantly FDG uptake. Several studies using either a cut-off of 5.5 mm^[39,40] or 6 mm^[41,42] in patients with chronic ischemic myocardial dysfunction could show that myocardial segments with wall thinning below the cut-off have a low likelihood of functional recovery after revascularization.

Overall these studies^[39-42] proved that EDWT has a good sensitivity and negative predictive value but only reasonable positive predictive value and poor specificity to predict functional recovery.

Figure 5 shows an example of a patient with a previous inferior myocardial infarction with severe thinning of the inferior myocardial wall segments.

Low dose dobutamine (\leq 10 μ g/kg per minute) stress CMR is another technique used to evaluate myocardial viability. At low doses, dobutamine supports coronary vasodilatation and increases myocardial contractility^[43]. Viable myocardium is distinguished by the identification of improved contractility under low dose dobutamine infusion. Several studies^[39-41,44-46] proved that a CMR-derived systolic wall thickening > 2 mm during low dose dobutamine stress is able to identify myocardial segments with functional recovery after revascularization. Accord-

Transmural extent of hyper-enhancement (%)	0	1-25	26-50	51-75	> 75
Improved contractility after revascularization (%)	78	60	42	10	< 2

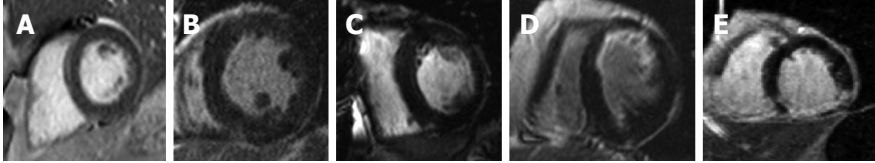


Figure 6 Late gadolinium enhancement imaging. Representative late gadolinium enhancement images of patients without scar (A), with a transmural extent of hyperenhancement of 1%-15% (B), 26%-50% (C), 51%-75% (D) and more than 75% (E) and the respective percentage of improved contractility according the study by Kim *et al*^[51].

ing to these studies^[39-41,44-46], the major strength of low dose dobutamine stress CMR is its high overall accuracy, specificity and positive predictive value.

LGE that was first applied by Kim *et al*^[47] has now become the gold standard for the evaluation of myocardial viability in ischemic heart disease. In nonviable tissue, the extracellular contrast agent spreads in a larger volume of distribution which results in delayed wash-out kinetics^[48]. Moreover, late enhancement imaging sequences suppress the signal derived from remote myocardium resulting in high image contrast. Hence, this technique allows the detection of even very small myocardial infarctions (≥ 0.7 g of myocardial mass)^[49]. In a meta-analysis by Romero *et al*^[50] LGE with a cut-off of < 50% transmural extent of scar tissue had a high sensitivity and a high negative predictive value to predict functional recovery. In patients with chronic ischemic heart disease the identification of viable myocardium is important to predict improvement of LVEF and survival after revascularization. In these patients the functional recovery was also linked to the transmural extent of scar^[51]. Kim *et al*^[51] could show that in segments without scar the functional recovery was 78% whereas in segments with a scar transmural extent of more than 75%, the likelihood of contractility improvement after revascularization was less than 2% (Figure 6). As illustrated in Figure 6, the ability to predict functional recovery in segments with an intermediate scar transmural extent between 1% and 75% ranged between 60% and 10%. In these patients with an intermediate scar transmural extent an additional low-dose dobutamine stress examination helps to identify segments that show a contractile reserve. A combined approach of LGE enhancement imaging and low-dose dobutamine stress imaging proved to be the optimal approach to predict recovery after revascularization^[44]. Therefore, in patients with wall motion abnormalities at rest the following algorithm as described by Nagel *et al*^[52] should be applied. LGE imaging should be used as first line imaging modality to identify patients without a scar who should undergo revascularization. Patients with more than 50% LGE transmural extent should be treated medically. In patients with less than 50% LGE transmural extent an additional low dose dobutamine stress CMR should be performed to detect patients with an improved contractility who are likely to benefit from revascularization. Whereas patients with less than 50% LGE transmural extent but without assessment of contractile reserve should

be treated medically. This algorithm indicates that in patients without evidence of LGE or with a LGE > 50% transmural extent, LGE imaging alone is sufficient. In case that an additional low dose dobutamine stress exam is required CMR allows to assess myocardial contractile reserve and LGE in a single comprehensive exam.

In patients with acute myocardial infarction and wall motion abnormalities CMR, Beek *et al*^[53] could also prove that LGE CMR is able to detect hibernating myocardium that is able to functionally recover. Further studies^[54,55] demonstrated that the transmural extent of delayed gadolinium enhancement correlates with the ability of functional improvement after acute myocardial infarction. Therefore, the distinction between reversible and irreversible dysfunctional myocardium in the acute setting after infarction also has a prognostic implication.

PROGNOSTIC ROLE OF LGE IN ISCHEMIC HEART DISEASE

Moreover, myocardial scar has been demonstrated to be the cause of malignant reentrant ventricular arrhythmias causing sudden cardiac death in patients after myocardial infarction^[56]. In patients with ischemic cardiomyopathy, Kwon *et al*^[57] revealed that a greater extent of myocardial scar was associated with a significantly increased mortality or the need for cardiac transplantation, improving further risk stratification. In patients undergoing ICD implantation with CAD, the extent of myocardial scarring visualized by LGE CMR was significantly associated with appropriate device therapy and identified a subgroup of CAD patients with an increased risk of life-threatening ventricular arrhythmias^[58].

FUTURE INDICATIONS FOR CMR IN PATIENTS WITH CAD

Novel methods like precontrast T_1 maps enable the detection of acute and chronic myocardial infarction^[59] and might represent a further field to establish the use of CMR as a key to tissue characterization. In a combined clinical protocol native T_1 mapping was suggested to reveal area at risk in ACS^[60,61].

Extracellular volume (ECV) maps as a CMR marker for myocardial fibrosis can be generated if pre and post contrast T_1 images are registered^[62]. In contrast to LGE

CMR, ECV is also able to visualize very early fibrotic changes^[63].

In the future, T1 mapping and ECV may provide more profound insights into fundamental disease processes of the myocardium. Both techniques might affect clinical decision making, but to date are not yet part of the routine work-up. Besides, the reproducibility of the results still needs to be shown in multi-centre studies^[64].

CONCLUSION

CMR is a non invasive imaging for the workup of patients with known or suspected CAD. It allows the detection of significant coronary stenoses in patients with acute and chronic chest pain. Moreover, it offers the unique opportunity to detect myocardial ischemia and viability or wall motion abnormalities and fibrosis in one examination. Novel techniques like T1 mapping and ECV will further expand the scope of application in the future.

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Positive airway pressure therapy for heart failure

Takao Kato, Shoko Suda, Takatoshi Kasai

Takao Kato, Department of Cardiology, Juntendo University School of Medicine, Tokyo 113-8421, Japan

Shoko Suda, Takatoshi Kasai, Department of Cardiology, Juntendo University School of Medicine and Cardio-Respiratory Sleep Medicine, Juntendo University Graduate School of Medicine, Tokyo 113-8421, Japan

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Correspondence to: Takatoshi Kasai, MD, PhD, Department of Cardiology, Juntendo University School of Medicine and Cardio-Respiratory Sleep Medicine, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan. kasai-t@mx6.nisq.net

Telephone: +81-3-38133111 Fax: +81-3-56890627

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Abstract

Heart failure (HF) is a life-threatening disease and is a growing public health concern. Despite recent advances in pharmacological management for HF, the morbidity and mortality from HF remain high. Therefore, non-pharmacological approaches for HF are being developed. However, most non-pharmacological approaches are invasive, have limited indication and are considered only for advanced HF. Accordingly, the development of less invasive, non-pharmacological approaches that improve outcomes for patients with HF is important. One such approach may include positive airway pressure (PAP) therapy. In this review, the role of PAP therapy applied through mask interfaces in the wide spectrum of HF care is discussed.

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Key words: Acute decompensated heart failure; Congestion; Continuous positive airway pressure; Non-

invasive positive airway pressure ventilation; Sleep disordered breathing

Core tip: Less-invasive, non-pharmacological approaches may improve outcomes for patients with heart failure, and the role of positive airway pressure therapy is discussed in this review.

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INTRODUCTION

Heart failure (HF) is a life-threatening disease and is a growing public health concern^[1,2]. The prevalence of HF has increased along with the aging of the general population^[3] and because of improved survival after acute myocardial infarction^[4,5]. Indeed, a better understanding of the pathophysiology and medical management of myocardial infarction means that such patients are living longer with damaged hearts, and many of them go on to develop HF^[5,6]. Despite recent advances in pharmacological management of HF, the morbidity and mortality from HF remain high^[4,5]. Therefore, non-pharmacological approaches to HF, including cardiac resynchronization therapy, and left ventricular (LV) assist devices, are increasingly utilized. However, most non-pharmacological approaches are invasive, have limited indication and are considered only for advanced HF. Accordingly, the development of less invasive, non-pharmacological approaches that may improve outcomes for patients with HF is important.

Positive airway pressure (PAP) therapy represents a potentially beneficial non-pharmacological approach to the management of HF. PAP therapy involves the maintenance of positive airway pressure through invasive

(applied with endotracheal intubation or tracheostomy) or non-invasive (applied without endotracheal intubation or tracheostomy) means. Because we focused on less invasive approaches to the management of HF, we confined our discussion to non-invasive positive pressure ventilation, including continuous positive airway pressure (CPAP), in which PAP is applied through nasal masks, oro-nasal masks and face masks^[7]. In the wide spectrum of HF care, PAP therapy can be used to improve oxygenation, decrease right ventricular (RV) afterload, alleviate hypoventilation and hypercapnia, improve lung and respiratory muscle functions, and normalize abnormal respiratory patterns. In this review, we discuss various types or modes, devices and equipment for PAP therapy, its effect on hemodynamics and respiration, and conditions for which PAP therapy should be considered in the care of HF patients. We also review the indications and evidence supporting the efficacy of PAP therapy in patients with HF.

EFFECT OF PAP ON HEMODYNAMICS AND RESPIRATION

All PAP therapies, which are considered for HF and mentioned later, adjunctively provide positive end-expiratory pressure. Therefore, the effect of PAP therapy, including positive end-expiratory pressure, on hemodynamics and respiration are described herein.

Effect on hemodynamics

PAP has several effects on hemodynamics. First, PAP diminishes systemic venous return and RV preload by increasing intrathoracic pressure^[8-10]. Second, PAP alters pulmonary total vascular resistance (PVR), which is the major determinant of RV afterload, *via* an alternation in lung volume^[11]. In the lungs, there are two types of vessels: the intra-alveolar vessels, which are compressed as lung volume increases, and the extra-alveolar vessels, which are exposed to dilating forces as lung volume increases. Thus, a change in total PVR is characterized by a U-shaped curve according to the alteration in lung volume (the lowest PVR can be observed in the lung volume around functional residual capacity)^[12]. For example, when lung volume increases from residual volume to functional residual capacity, the effects of this increased volume on extra-alveolar vessels will predominate, and thus, vascular capacitance will increase. Consequently, total PVR will decrease. When the lung volume continues to increase from functional residual capacity to total lung capacity, the effects of this further increased volume on intra-alveolar vessels will predominate; vascular capacitance will therefore decrease, and total PVR will increase^[12,13]. Although PAP without an excessive shift in lung volume does not cause a clinically important increase in RV afterload^[12], it is possible that RV afterload can be increased by PAP^[14]. Third, a decrease in RV preload (decrease in systemic venous return to RV) and an increase in RV afterload (increase in PVR) lead to a reduction in pul-

monary venous return and limitations in LV inflow and filling. In addition, in cases with increased RV afterload, RV dilatation with a septal shift toward the LV can occur. This further limits the LV filling and causes reductions in cardiac output and overall organ perfusion^[8,10,14-16]. Fourth, increased intrathoracic pressure relative to atmospheric pressure causes a pressure difference between the extrathoracic and intrathoracic cavities because most of the systemic circulation is at atmospheric pressure, which is lower than that of the LV and thoracic aorta^[17]. Therefore, PAP therapy can reduce RV preload and increase RV afterload, whereas PAP therapy reduces LV preload and afterload (Figure 1). In general, subjects without HF are predominantly preload-dependent^[18]. Therefore, in subjects without HF or in patients who manifest preload-dependent LV function, such as those with RV infarction or hypovolemia, a reduction in cardiac preload with PAP therapy may decrease cardiac output to a greater degree than decreasing afterload and its related increase in cardiac output^[14].

Conversely, a failing heart is more sensitive to decreased afterload, and patients with HF are usually hypervolemic and thus insensitive to decreased preload. Therefore, patients with HF are predominantly afterload-dependent, and cardiac output can be increased by PAP therapy in those patients. Nevertheless, in HF patients, the preload- and afterload-dependent status will determine the cardiac output responses (increase or decrease) (Figure 1).

Effect on respiration

PAP also has several effects on respiration in HF. First, PAP maintains alveolar pressure and prevents the alveoli from collapsing at the end of expiration and thus improves gas exchange and oxygenation through the recruitment of alveolar units, counterbalance of hydrostatic forces leading to pulmonary edema, and maintenance of airway patency^[19-22]. Particularly in HF patients with pulmonary congestion in whom lung compliance is impaired, PAP induces recruitment of collapsed alveoli, reversal of atelectasis, and induces a fluid shift from the alveoli and the interstitial space to the pulmonary circulation, consequently decreasing the amount of intrapulmonary shunting and improving oxygenation^[21,23]. Second, PAP can reduce respiratory muscle load and the work of breathing^[24-26] and can improve lung function through lung inflation and maintenance of functional residual capacity^[27]. Third, PAP prevents upper airway narrowing and collapse and thereby functions as a “pneumatic splint”^[28-30]. This is highly effective in the treatment of sleep-disordered breathing (SDB)^[31], which is frequently observed in patients with HF^[32]. Fourth, some PAP therapy provides pressure support during inspiration to maintain ventilation. This is particularly important in HF patients with hypoventilation. Fifth, if hypoxic pulmonary vasoconstriction occurs due to hypoxia in association with acute decompensated HF (ADHF) or HF accompanied by chronic obstructive lung disease (COPD)

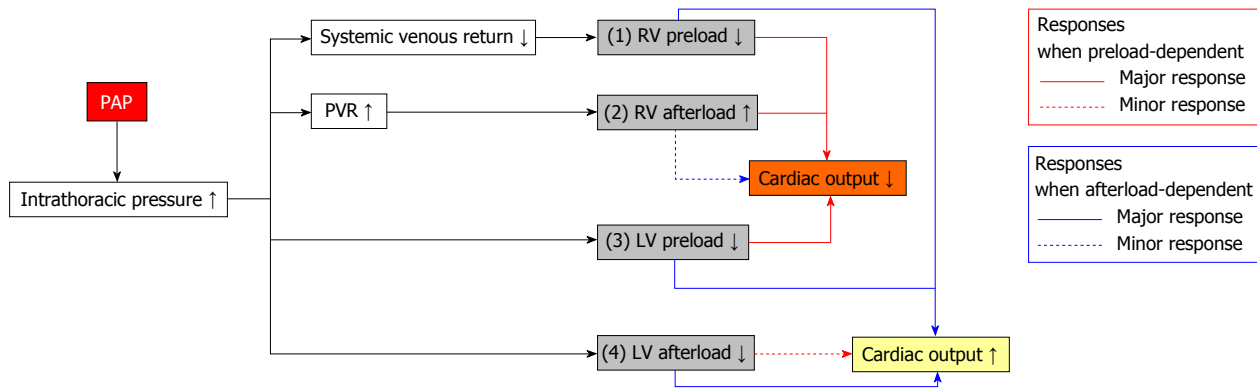


Figure 1 Effects of positive airway pressure on hemodynamics. First, PAP decreases systemic venous return and RV preload by increasing intrathoracic pressure; Second, PAP increases PVR by increasing lung volume. Thus, it is possible that RV afterload can be increased by PAP; Third, a decrease in RV preload and an increase in RV afterload lead to reductions in pulmonary venous return and limitations of LV inflow, filling and preload; Fourth, increased intrathoracic pressure relative to atmospheric pressure causes a pressure difference between the intrathoracic and extrathoracic cavities. Therefore, PAP may decrease LV afterload. In subjects without HF who are generally preload-dependent or in HF patients who manifest preload-dependent reduction, decreased RV and LV preload in addition to the increase in RV afterload may cause a net decrease in cardiac output, whereas a decrease in LV afterload may cause a minor response toward increasing cardiac output. Conversely, patients with HF are more sensitive to decreased afterload and are thus predominantly afterload-dependent. PAP therapy causes a net increase in cardiac output through decreases in RV preload, LV preload and afterload, whereas an increase in RV afterload may cause a minor response toward decreasing cardiac output. LV: Left ventricular; PAP: Positive airway pressure; PVR: Pulmonary vascular resistance; RV: Right ventricular; HF: Heart failure.

Table 1 Summary of equipped functions of each type/mode of positive airway pressure

	CPAP	Bi-level PAP	VAPS	ASV
Positive end-expiratory pressure	+	+	+	+
Pressure support during inspiration	-	+	+	+
Guarantee of tidal volume or minute ventilation	-	-	+	-
Servo-control of ventilation	-	-	-	+
Automated control of pressure level during expiration	+	-	-	+
Backup ventilation	-	\pm^1	$^{+2}$	$^{+2}$

¹Bi-level PAP devices that are only capable of spontaneous mode cannot provide back-up ventilation; ²Most devices automatically provide a set backup ventilation rate based on their VAPS or ASV algorithm. ASV: Adaptive servo-ventilation; Bi-level PAP: Bi-level positive airway pressure; CPAP: Continuous positive airway pressure; VAPS: Volume assured pressure support.

and SDB, PAP can attenuate the increase in PVR by improving oxygenation through the abovementioned effect and by alleviating vasoconstriction. Consequently, such attenuation of increased PVR can be associated with improving hemodynamics. Finally, considering that the short-term servo-control of ventilation using adaptive servo ventilation (ASV) during wakefulness reduced muscle sympathetic nervous system activity in patients with chronic HF^[33,34], keeping ventilation consistent with ASV may provide further beneficial effects on hemodynamics that are independent of the effects of PAP^[35].

TYPES/MODES OF PAP IN HF

TREATMENT

Several types or modes of PAP therapy can be considered for HF. Although each type or mode has different purposes, all of them apply positive pressure to the air-

way. In particular, all of them provide positive end-expiratory pressure. Thus, the benefits from the individual modes overlap. In this section, the types and modes of PAP generally applied for HF are described (Table 1).

CPAP

CPAP is the most widely used type/mode of PAP therapy in patients with HF. It provides a constant level of positive pressure to maintain airway patency during spontaneous breathing (Figure 2A). Because CPAP only provides a constant level of pressure during the entire respiratory cycle and because CPAP does not separately increase pressure during inspiration and thus does not directly support ventilation, sometimes CPAP is not classified as a form of non-invasive positive pressure ventilation. However, the International Consensus Conference in Intensive Care Medicine^[7] defined non-invasive positive pressure ventilation as any form of PAP support applied without endotracheal intubation, in which the pressure is generated by the respiratory muscles only with a spontaneous support modality, such as CPAP, or by the ventilator only or by the ventilator and the respiratory muscles. Thus, we classify CPAP as non-invasive positive pressure ventilation unless otherwise indicated.

In general practice, CPAP is most commonly used for the management of SDB *via* specifically manufactured CPAP devices for home care. Some of these CPAP devices are designed to detect various degrees of upper airway obstruction and then adjust the pressure level to keep the airway open. Some of these systems can also provide information about the residual apneas or hypopneas while patients are on CPAP (*i.e.*, automated CPAP) (Figure 1B)^[36,37]. Although treatment with automated CPAP improves patient satisfaction and compliance in a subset of patients with obstructive sleep apnea (OSA), the routine use of automated CPAP for OSA treatment provides limited benefit^[38-40]. Furthermore, although

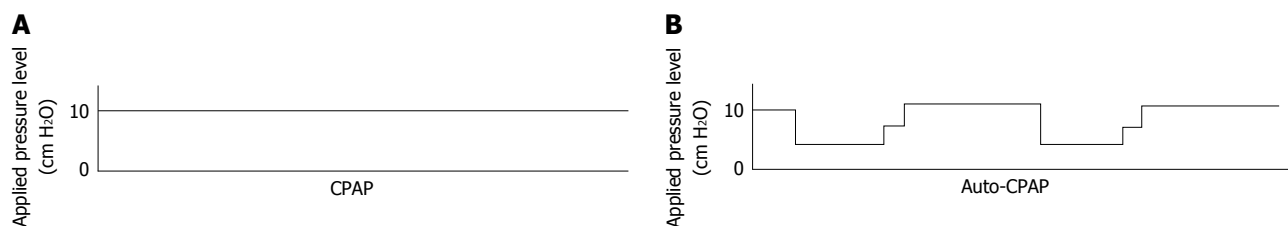


Figure 2 Differences between continuous positive airway pressure and automated continuous positive airway pressure. A: CPAP provides a constant level of positive pressure to the airway during spontaneous breathing; B: Automated CPAP devices are designed to detect various degrees of upper airway obstruction and consequently adjust the pressure level to keep the airway open. CPAP: Continuous positive airway pressure.

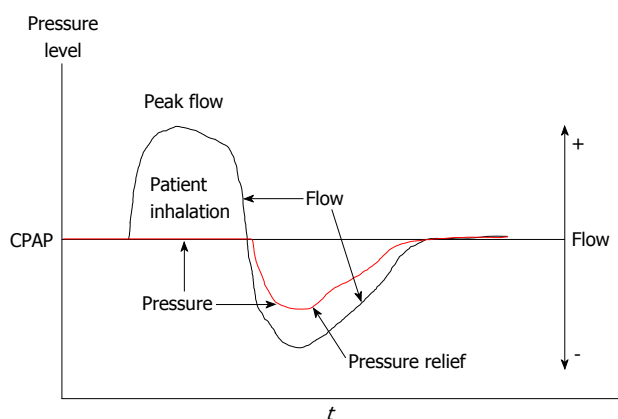


Figure 3 Algorithm of early expiratory phase pressure relief. The pressure is lowered in the early phase of expiration to enhance comfort, but the pressure returns to the critical pressure needed to keep the airway open before the next inspiration. CPAP: Continuous positive airway pressure.

more recent automated CPAP devices have an algorithm to detect central respiratory events, the accuracy of this algorithm remains to be elucidated. Thus, current guidelines do not recommend automated CPAP devices for the diagnosis of SDB or for the treatment of patients with HF, in which central respiratory events frequently coexist with OSA^[41].

Some patients who cannot tolerate CPAP complain of difficulty while exhaling against the airway pressure generated by the CPAP device^[42,43] especially in patients whose therapeutic pressure needed to eliminate OSA is fairly high (*e.g.*, > 10 cm H₂O). To resolve this issue, some CPAP devices use specific algorithms, such as early expiratory phase pressure-relief (Figure 2). Using these algorithms, the pressure is lowered in the early phase of expiration to enhance comfort, but the pressure returns to the critical pressure needed for keeping airway open before the next inspiration. Early expiratory pressure relief can be applied in combination with other modes of PAP therapy. Because patients with HF do not require such high pressures, even those with OSA, and because high-pressure CPAP might reduce cardiac output in some cases of HF, a pressure-relief algorithm is rarely used when treating HF patients.

Bi-level PAP

Bi-level PAP provides two fixed levels of PAP: a higher level of pressure during inspiration (inspiratory posi-

tive airway pressure (IPAP) and a lower level of pressure during expiration [expiratory positive airway pressure (EPAP)]. Its major difference from CPAP is that it provides pressure support during inspiration (Figure 3). The level of pressure support is determined as a difference between IPAP and EPAP, and the level of IPAP plays an important role in unloading respiratory muscles, reducing the work of breathing, controlling obstructive hypopnea or flow limitation, maintaining alveolar ventilation, and reducing the partial pressure of carbon dioxide (PaCO₂). EPAP produces respiration and hemodynamic effects that are similar to those provided by CPAP. In addition, most bi-level PAP devices have several modes for back-up ventilation, such as spontaneous breathing (S-mode), timed back-up ventilation, and spontaneous breathing with timed back-up ventilation (ST-mode). Bi-level PAP with S-mode can be used for patients who require high-pressure CPAP to control OSA or for those who cannot tolerate exhaling against high pressure CPAP^[44]. However, patients with HF generally do not require high pressure, even those with OSA. Thus, the indication for bi-level PAP with S-mode in patients with HF is quite limited. When using CPAP, the airway pressure is increased at end-expiration but decreased during inspiration (Figure 4); because the net cardiac unloading effects during the respiration cycle are greater in bi-level PAP than in CPAP in association with an increased pressure level during inspiration and its unloading effects, bi-level PAP may be a better option for the treatment of HF^[14,45]. In the care of HF, the purposes of treatment with bi-level PAP include the following: (1) reducing hypercapnia in some patients with acute decompensated HF or those with co-existing COPD and HF or those with obesity hypoventilation syndrome (OHS) and its related HF; (2) keeping ventilation consistent with a constant pressure support; and (3) back-up ventilation in patients with central sleep apnea (CSA). In patients with CSA, hypocapnia related to hyperventilation due to pulmonary congestion plays an important role in the development and maintenance of CSA^[46]. Bi-level PAP sufficiently promotes ventilation and can reduce the carbon dioxide levels to below the apneic threshold during sleep.

Volume-assured pressure support

Volume-assured pressure support (VAPS) is an advanced mode of bi-level PAP developed for the treatment of pa-

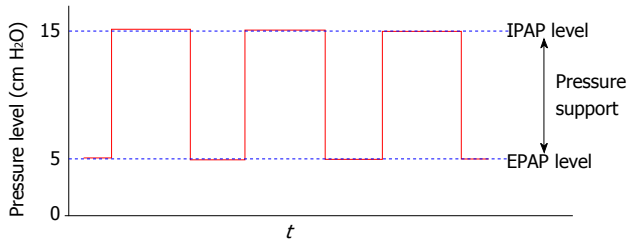


Figure 4 Bi-level positive airway pressure. Bi-level PAP provides two fixed levels of PAP, a higher level of pressure during inspiration (*i.e.*, IPAP) and a lower level of pressure during expiration (*i.e.*, EPAP), and thus provides pressure support during inspiration. EPAP: Expiratory positive airway pressure; IPAP: Inspiratory positive airway pressure; PAP: Positive airway pressure.

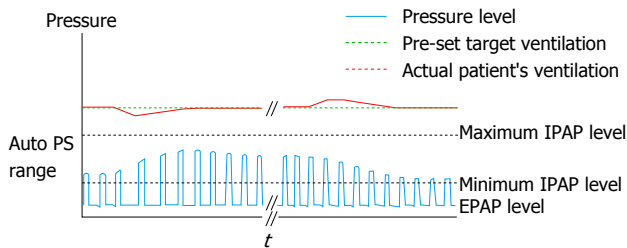


Figure 6 Volume assured pressure support. Using the volume assured pressure support mode, the device alters the level of pressure support from the minimum to maximum levels to maintain the pre-set ventilation or pre-set target tidal volume. This figure shows algorithm based on ventilation. EPAP: Expiratory positive airway pressure; IPAP: Inspiratory positive airway pressure; PS: Pressure support.

tients with hypoventilation and hypercapnia^[47-51]. In VAPS mode, the device alters the level of pressure support (*i.e.*, IPAP level) to maintain a pre-set target tidal volume. Devices with newer VAPS modes alter the respiratory rate in addition to the level of pressure support to maintain a pre-set minute ventilation. Nevertheless, VAPS mode guarantees a delivered tidal volume or minute ventilation despite patients' variable breathing effort, airway resistance, and lung or chest wall compliance (Figure 5).

ASV

ASV is an advanced mode of bi-level PAP developed for the treatment of Cheyne-Stokes respiration with CSA in patients with HF^[52]. It is also used in the treatment of other forms of CSA, such as idiopathic CSA, CPAP-emerged CSA and opioid-induced CSA^[53,54]. ASV devices automatically provide altering pressure support for each inspiration, ranging from a pre-set minimum level to a pre-set maximum level, to maintain moving target ventilation (determined based on volume or flow) determined by the patient's current breathing in addition to the back-up ventilation with variable respiratory rates (*i.e.*, servo-control of ventilation) (Figure 6). In addition, more recent devices provide altering EPAP levels that are sufficient for the control of upper airway narrowing or collapse, using an algorithm that is similar to that used by automated CPAP. The goals of ASV are to stabilize abnormal breathing patterns (*i.e.*, CSA with Cheyne-Stokes respiration) and to maintain the PaCO₂ level to prevent

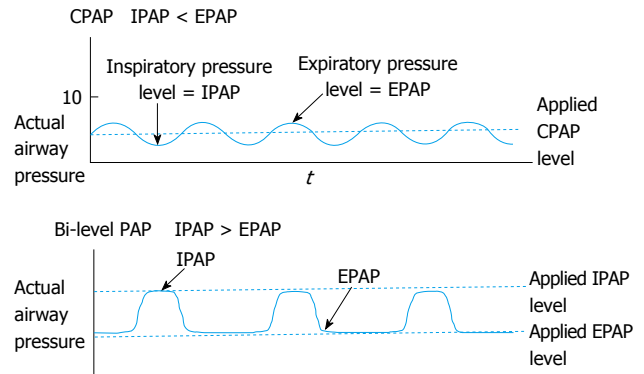


Figure 5 Differences in actual airway pressure between continuous positive airway pressure and bi-level positive airway pressure. While on CPAP, although a constant CPAP level is applied, actual airway pressure is not constant and oscillates. During inspiration, actual airway pressure decreases below the applied CPAP level, whereas during expiration, actual airway pressure increases above the applied CPAP level. Thus, the inspiratory pressure level in the airway (*i.e.*, IPAP) is lower than the expiratory pressure level in the airway (*i.e.*, EPAP). Conversely, while on bi-level PAP, the actual airway pressure increases during inspiration due to pressure support. Thus, IPAP is greater than EPAP according to the level of pressure support. CPAP: Continuous positive airway pressure; EPAP: Expiratory positive airway pressure; IPAP: Inspiratory positive airway pressure; PAP: Positive airway pressure.

hypocapnia, which can trigger apnea reentry cycles^[52] in addition to keeping the upper airway open (Figure 7).

There are two major ASV devices. In both products, pressure support is dynamically adjusted breath-to-breath as necessary to ensure that the patients' actual ventilation matches the target value in addition to the auto-titration of EPAP to maintain airway patency. The main points of difference are the mechanics used to assess the breathing status and to determine the target level. One type of device uses volume-targeted ASV, which sets a minute-ventilation target that is 90% of the recent average minute volume from a 3-min collection period and tries to maintain ventilation at the target level^[52]. The other device uses flow-targeted ASV, which monitors the peak inspiratory flow of the patient over a recent moving 4-min window, calculating an average peak flow at every point within this window to set a target peak flow. It compares these data to an internal target and maintains a target peak inspiratory flow^[55]. The other minor differences between volume-triggered and flow-triggered ASV devices are summarized in Table 2.

DEVICES, INTERFACE AND ADDITIONAL EQUIPMENT

In general, the equipment necessary for PAP therapy includes devices that provide PAP, tubing and several types of patient interfaces^[56]. Ventilators that are used in standard critical care for invasive ventilation can also be used for non-invasive PAP therapy with specific patient interfaces. However, a few types of ventilators have specifically been designed to provide PAP noninvasively and are generally used during the acute phase of HF. Most of these non-invasive ventilators employ several of the

Table 2 Adaptive servo-ventilation devices

Volume-triggered ASV		Flow-triggered ASV
Manufacturer	ResMed	Philips-Respironics
Target	90% of previous average ventilation (moving time window)	90% of average peak flow (moving time window)
EPAP/EEP	EEP automatically adjusted between min and max (4-20 cm H ₂ O) Cannot select auto EEP without PS	EPAP automatically adjusted between min and max (4-25 cm H ₂ O)
IPAP	Max pressure up to 30 cm H ₂ O IPAP changes within pre-set PS range from min (can be 0) to max Max PS can be limited by maximum pressure and current EEP	Max pressure up to 25 cm H ₂ O IPAP changes within pre-set PS range from min (can be 0) to max (21 cm H ₂ O) Max PS can be limited by pre-set maximum pressure and current EPAP level
Backup rate	Automatic 15 ± α breaths/min	Auto rate Fixed rate
Pressure wave form	Saw-tooth	Square shape
Inspiratory time	Automatic	Automatic in auto rate mode Set in manual rate mode

ASV: Adaptive servo-ventilation; EEP: End-expiratory pressure (*i.e.*, = EPAP); EPAP: Expiratory positive airway pressure; IPAP: Inspiratory positive airway pressure; PS: Pressure support.

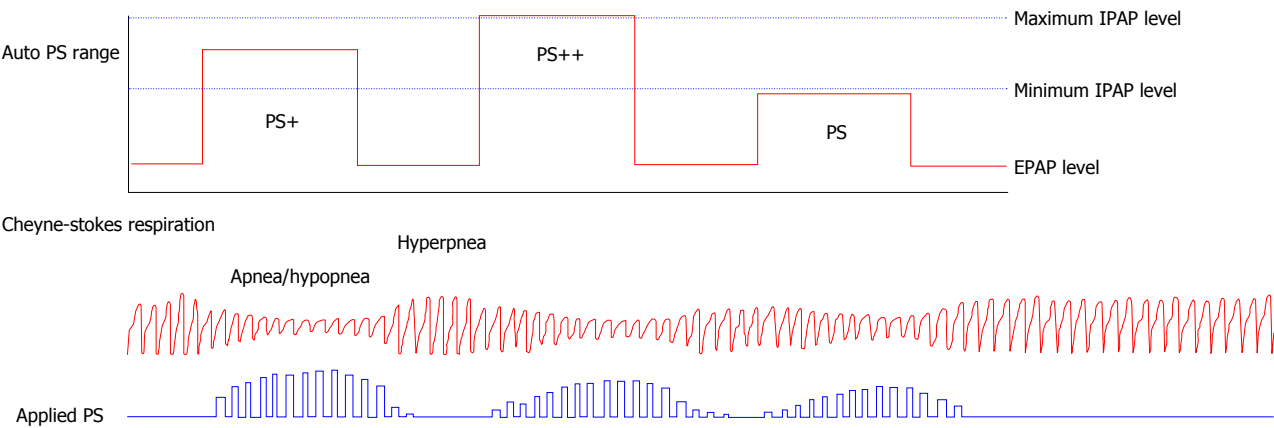


Figure 7 Adaptive servo-ventilation. Adaptive servo-ventilation devices automatically provide altering pressure support for each inspiration, ranging from a pre-set minimum level to a pre-set maximum level, to maintain moving target ventilation (determined based on volume or flow) determined by the patient's current breathing in addition to the back-up ventilation with variable respiratory rates. This stabilizes the abnormal breathing pattern (*i.e.*, cheyne-stokes respiration) and maintains the PaCO₂ levels to prevent hypocapnia, which can otherwise trigger apnea reentry cycles. EPAP: Expiratory positive airway pressure; IPAP: inspiratory positive airway pressure; PaCO₂: Arterial partial pressure of carbon dioxide; PS: Pressure support.

modes of PAP therapy mentioned earlier and can be used in many situations and conditions during the acute phase of HF. In addition, there are several smaller and more simplified devices that can provide only one or two modes for less intensive care in the general cardiology wards or for home care in patients with sub-acute or chronic phases of HF.

In terms of interface, various masks have been used for PAP therapy for HF; these include nasal masks, nasal pillows, oro-nasal (full-face) masks that cover the nose and mouth, and face (total-face) masks that cover the entire face^[57], all of whose actual attachment portions to the face are made of silicone or other soft rubber-like materials to achieve a tighter air seal^[58]. In acute HF, a disposable oro-nasal mask or face mask is usually used. In the home care setting, the choice of mask is the most important issue for patient comfort and tolerance to PAP therapy. Poorly fitted masks decrease the efficacy, compliance and adherence to PAP therapy. In addition to the mask itself, headgear or straps are used as a harness.

Overly tight headgear may worsen the air leak and interfere with patient comfort and compliance.

Some patients receiving long-term PAP therapy complain of nasal oro-nasal dryness while on PAP devices^[59]. For such patients, a heated humidifier can be used to maintain compliance with therapy^[60]. One possible disadvantage of the use of heated humidifier includes the accumulation of condensate inside the tube, which can cause a decrease in inspiratory pressure and a delay of triggering when bi-level PAP is used. Condensation inside the tube is also frequently observed during the winter in the home care setting^[61]. To resolve such condensate issues, heated tubing systems containing copper wire are now available for clinical use.

CONTRAINDICATION TO PAP THERAPY

There are several absolute contraindications to PAP therapy, such as the presence or absence of anatomic abnormalities for attaching the interface and recent

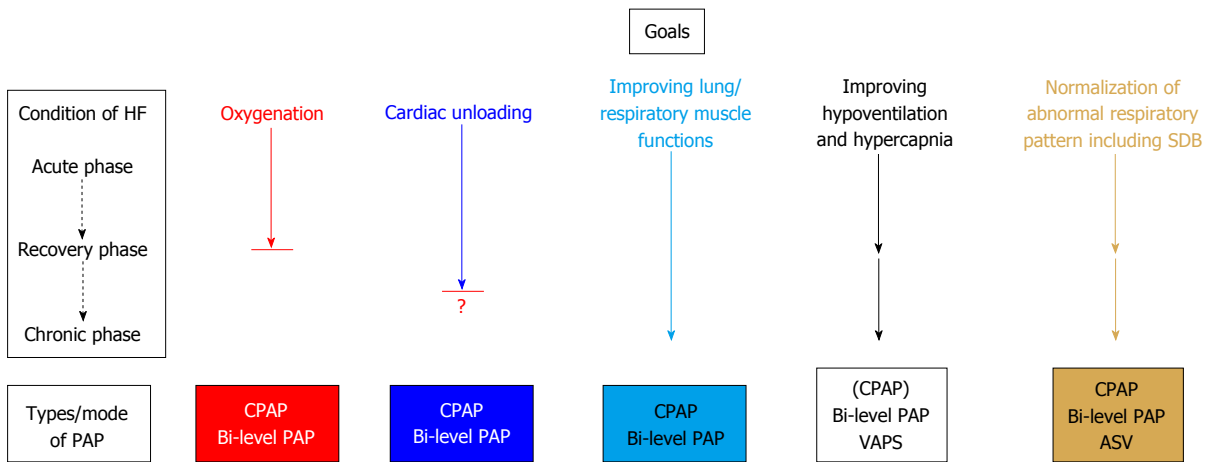


Figure 8 Importance of each goal of positive airway pressure therapy according to different heart failure conditions. In the wide spectrum of HF care, PAP therapy is used to help oxygenation, provide relief from cardiac load, improve lung and respiratory muscle function, reduce hypoventilation and hypercapnia, and normalize abnormal respiratory patterns, including SDB. The importance of each goal can differ according to the condition of HF. Improving oxygen is one of the most important goals in the acute phase. However, after recovery from acute decompensation, this goal becomes less important or is no longer considered. Providing cardiac unloading is another important goal in the acute phase to the recovery phase. However, the importance of this goal remains to be elucidated after recovery from acute decompensation. Improving lung and respiratory muscle function is sometimes important in the acute phase and after recovery. Improving hypoventilation is important in cases with hypercapnia in the acute phase. In addition, HF patients with hypoventilation and daytime hypercapnia can be treated by PAP therapy in the recovery or chronic phase. Normalization of abnormal respiratory patterns, particularly SDB suppression, is sometimes important in the recovery phase and is most important in the chronic phase. The specific types/modes of PAP that should be used differ according to each therapeutic purpose. ASV: Adaptive servo-ventilation; CPAP: Continuous positive airway pressure; HF: Heart failure; PAP: Positive airway pressure; SDB: Sleep disordered breathing; VAPS: Volume assured pressure support.

airway or gastrointestinal surgery. Relative contraindications include the need for the patient to be capable of airway protection, an increased risk of aspiration, swallowing impairment, excessive secretions, frequent coughing, severe hypoxemia (*i.e.*, $\text{PaO}_2/\text{FiO}_2 < 75$), acidemia, multiorgan failure, respiratory arrest, inability to fit the mask, or poorly motivated patient or family^[62,63]. There is controversy regarding use of PAP therapy for patients with cardiogenic shock and hemodynamic instability^[18]. In the care of HF, PAP therapy should be administered with caution in patients with severe right-side HF accompanied by severe liver congestion or cirrhosis, patients with hypertrophic obstructive cardiomyopathy and patients with severe aortic valvular heart disease because reductions in venous return to the heart may worsen liver congestion, ascites and edema, and because reductions in LV preload and afterload may cause further reduction in cardiac output unless these patients also have severe pulmonary congestion.

COMPLICATIONS

PAP therapy is generally safe, and only a few major complications can occur, including aspiration pneumonia^[64,65], hypotension as a consequence of reduction of preload and afterload (see “Effect of positive airway pressure on hemodynamics”), and rarely, pulmonary barotraumas in association with excessive pressures. Because excessive pressures are not applied for patients with HF due to the risk of adverse reduction in cardiac output, actual applied pressures are much lower than such excessive pressure levels.

However, minor complications related to masks or

pressures and air flows can occur. Fitting the mask too tightly for long periods of time may result in skin damage and ulceration, particularly around the nasal bridge^[66]. Once established, such wounds may require artificial skin grafts, and of course, mask re-fitting should be considered. Furthermore, patients undergoing long-term PAP therapy with masks might have global facial flattening^[67]. However, this may be a specific complication for children. Discomfort associated with pressures and air flows are common and can include dryness, pain in the nose or mouth and pneumophagia, all of which are usually resolved *via* the use of a humidifier or by a decrease in pressure levels.

CONDITIONS IN WHICH PAP THERAPY IS CONSIDERED FOR HF

In the wide spectrum of HF care, PAP therapy is used to improve oxygenation, reduce cardiac load, improve lung and respiratory muscle function, alleviate hypoventilation and hypercapnia, and normalize abnormal respiratory patterns, including SDB (Figure 8). In this section, specific conditions in which PAP therapy is frequently considered in the care of HF are described (Table 3).

Acute decompensated HF

Guidelines for ADHF generally recommend the use of PAP therapy if patients have breathing difficulty, signs of pulmonary edema, or hypoxia despite supplemental oxygen (Table 4)^[5,68-71]. For patients with ADHF, the purposes of PAP therapy include augmentation of oxygenation through recruitment of collapsed alveoli, reversal of atelectasis, and induction of fluid shifts back from the alveoli

Table 3 Possible indication of each type/mode of positive airway pressure for each condition

	CPAP	Bi-level PAP	VAPS	ASV
Acute decompensated heart failure	o ¹	o	? ²	?
Chronic HF with OSA	o	o	Δ ³	Δ ⁴
Chronic HF with CSA	Δ ⁵	o	?	o
HF following acute decompensation	Δ ⁶	o	?	Δ ⁷
Chronic HF without SDB	x	?	?	Δ ⁸
HF with hypoventilation (acute)	Δ ⁹	o	o	x
HF with hypoventilation (chronic)	Δ ⁹	o	o	?

¹Bi-level PAP with S-mode for accompanying OSA; ²Indicates that no clear data are available; ³Can be used if accompanied by hypoventilation; ⁴Can be used if Cheyne-Stokes respiration coexists; ⁵Can be used if CSA is alleviated; ⁶Can be used if OSA exists; ⁷Can be used if CSA exists; ⁸ASV may be useful for chronic HF patients with apnea-hypopnea index < 20 including those without SDB; ⁹Can be used if hypoventilation is associated with OSA. ASV: Adaptive servo-ventilation; Bi-level PAP: Bi-level positive airway pressure; CPAP: Continuous positive airway pressure; CSA: Central sleep apnea; HF: Heart failure; OSA: Obstructive sleep apnea; SDB: Sleep disordered breathing; VAPS: Volume assured pressure support.

and the interstitial space to the pulmonary circulation, reducing respiratory muscle load and the work of breathing, and stabilizing hemodynamics *via* cardiac unloading.

In patients with ADHF, PAP therapy is usually administered by specifically designed ventilators for non-invasive PAP for acute or intensive care. The selection of modes (usually, CPAP or bi-level PAP) is dependent on whether patients require pressure support to ventilate appropriately. For example, patients with hypercapnia or respiratory muscle fatigue may require bi-level PAP. Otherwise, CPAP is used because most data suggest that there are no obvious clinical benefits to the use of bi-level PAP over CPAP^[72,73]. In Japan, ASV is sometimes used for patients with ADHF, especially in institutions where specifically designed ventilators for non-invasive PAP for acute or intensive care are not available. The merits of using ASV for patients with ADHF may include the fact that devices for ASV are small, handy and mobile; ASV can be started in the emergency room, which allows PAP to be applied quite immediately upon presentation to the hospital; and ASV may synchronize patients' respiration more easily than typical bi-level PAP. However, these potential merits of ASV remain to be confirmed. It should be noted that ASV devices do not provide raw wave forms of parameters related to respiration, whereas specifically designed ventilators for non-invasive PAP for acute or intensive care do provide these data.

PAP therapy improves hemodynamics, respiratory function and oxygenation in patients with pulmonary edema in association with ADHF when compared with oxygen therapy alone^[74-79]. Moreover, the use of PAP therapy in randomized prospective trials was associated with lower rates of intubation and improved 30-d mortality compared with oxygen therapy alone^[74,76,77,79]. Thus, PAP therapy for ADHF is the universal standard.

SDB

SDB is frequently observed in patients with HF. In gen-

eral, two types of SDB, OSA and CSA, can be observed in HF patients. Typically, CSA in HF patients is usually observed as Cheyne-Stokes respiration, which is a form of periodic breathing characterized by a crescendo-decrescendo pattern of breathing followed by central apnea or hypopnea^[80].

OSA results from upper airway collapse and predisposes patients to the development and progression of HF *via* several mechanisms. For example, in patients with OSA, the blood pressure is frequently elevated as a result of overactivation of the sympathetic nervous system. Such high blood pressure may contribute to the development of HF in association with the direct deleterious effects of sympathetic overactivity. In addition, the generation of exaggerated negative intrathoracic pressure during obstructive apneas increases cardiac loads. Conversely, CSA appears to arise secondary to HF. In general, HF patients are likely to have chronic hyperventilation due to stimulation of the pulmonary vagal irritant receptors by pulmonary congestion and to increased chemosensitivity, which is characteristic of HF patients with CSA^[46,81] and consequently results in hypocapnia. When PaCO₂ falls below the apneic threshold because of an increase in the apneic threshold during transition from wakefulness to sleep, CSA ensues^[46,81]. Apnea persists until PaCO₂ rises above the apneic threshold; then, ventilation will resume; ventilatory overshoot occurs, and PaCO₂ will decrease below the apneic threshold in association with arousal during the ventilatory phase and increased chemosensitivity. This could also contribute to the pathogenesis of CSA with a Cheyne-Stokes respiration pattern by facilitating ventilatory overshoot and undershoot. Once triggered, the pattern of Cheyne-Stokes respiration will be sustained by the combination of increased respiratory chemoreceptor drive, pulmonary congestion, arousals, and apnea-induced hypoxia, which cause oscillations in PaCO₂ above and below the apnea threshold^[46,81]. Nevertheless, CSA is also characterized by apnea, hypoxia, and increased sympathetic nervous activity and, when present in HF, is associated with an increased risk of death^[46,81,82].

In patients with chronic HF, treatment of SDB alleviates underlying cardiac dysfunction. The standard treatment for OSA in patients with HF is CPAP. CPAP prevents upper airway narrowing and collapse and works as a "pneumatic splint"^[28-30], thereby preventing obstructive apneas and hypopneas. It was reported that one night of CPAP resolved negative intrathoracic pressure swings in association with obstructive respiratory events and reductions in nocturnal blood pressure and heart rate^[46,83]. Thus, HF patients with OSA may benefit from cardiac unloading by suppressing OSA *via* CPAP. Independent of OSA suppression, CPAP promotes reductions in LV preload and afterload in patients with HF. In fact, many studies regarding CPAP therapy for chronic HF patients demonstrated an improvement in LV systolic function in association with reductions in sympathetic nervous system activity and associated reductions in systemic arterial blood pressure and heart rate^[46,84-87]. In terms of long-term clinical outcomes, two observational studies

Table 4 Recommendations for oxygen and bi-level positive airway pressure therapy for acute decompensated heart failure

Guidelines	Oxygen	PAP therapy
ACC/AHA (2009 updated)	To relieve symptoms related to hypoxemia: Class I, level C	NA
ACCF/AHA (2013)	NA	NA
HFSA (2010)	Hypoxia+: Class I, level C Hypoxia-: Class III, level C	Dyspnea+ or pulmonary edema+: Class I, level A
ESC (2012)	Hypoxemia+ (SaO ₂ < 90% or PaO ₂ < 60 mmHg): Class I, level C	Dyspnea+ or pulmonary edema+ or RR > 20/min: Class IIa, level B SBP < 85 mmHg: Class III
JCS (2011)	Hypoxia+ (to keep SaO ₂ > 95%, PaO ₂ > 80 mmHg): Class I, level C	Not responding to oxygen: Class I, level A

ACC: American college of cardiology; ACCF: American college of Cardiology foundation; AHA: American heart association; ESC: European Society of Cardiology; HFSA: Heart failure society of America; JCS: Japanese Cardiology Society; NA: Not available; PaO₂: Arterial partial pressure of oxygen; RR: Respiratory rate; SaO₂: Oxyhemoglobin saturation; SBP: Systolic blood pressure; PAP: Positive airway pressure.

in chronic HF patients indicated that CPAP therapy for OSA results in a trend towards reduced mortality or a significant reduction in the composite endpoint of mortality and rehospitalization^[88,89]. In addition, in one of those studies, the hospitalization-free survival rate in patients administered CPAP therapy was significantly higher in the more compliant group than in the less compliant group^[89]. Therefore, good compliance with long-term CPAP therapy may provide better clinical outcomes in chronic HF patients with OSA.

Because HF patients with CSA have associated pulmonary congestion and increased LV filling pressures, CPAP has been applied to improve pulmonary congestion and increased LV filling through the cardiac unloading. However, studies regarding the effects of CPAP on the suppression of CSA in chronic HF patients produced inconsistent results, most likely due to the differences the application of CPAP. If CPAP was applied for a short period of time (*e.g.*, 1 night) at low pressure (*e.g.*, 5 cm and 7.5 cm H₂O), CSA was not alleviated^[90,91]. However, if CPAP was applied for longer periods (*e.g.*, 7 d) at high pressure (8–12.5 cm H₂O), the severity of CSA decreased by > 50%^[92–96]. In addition, CPAP with gradual titration alleviated CSA and was accompanied by an increase in PaCO₂^[46,95,97,98], reduction in sympathetic nervous system activity^[99], and improvements in respiratory muscle function^[100] and LV systolic function for 1–3 mo^[46,95–98]. In terms of long-term clinical outcome, one small-randomized trial^[97] showed that in chronic HF patients with CSA, CPAP produced a trend^[90] toward a better outcome, and a sub-group of patients compliant with CPAP had significantly better outcomes. However, a large-scale randomized controlled study in chronic HF patients with CSA failed to demonstrate the benefits of CPAP in terms of long-term clinical outcomes (mean follow-up duration, 2-years)^[101]. A post hoc analysis of this study suggested that patients whose apnea-hypopnea index (AHI) decreased below 15 in response to CPAP at 3 mo (*i.e.*, CPAP responder) had significantly better long-term clinical outcomes compared with the control groups. This implied that in approximately 50% of chronic HF patients, CPAP therapy suppressed CSA, but PAP therapy, which may suppress CSA more effectively and constantly,

should be the focus.

One such PAP therapy is bi-level PAP^[52]. A small randomized controlled trial comparing 10 HF patients with CSA on bi-level PAP without backup ventilation (*i.e.*, S-mode) and standard medical therapy versus 11 HF patients with CSA on standard medical therapy alone showed significant reduction in the AHI from 28.3 ± 12.3/h to 5.2 ± 3.8/h with one night of bi-level PAP with S-mode and significant improvement in LV ejection fraction at 3 mo with bi-level PAP with S-mode (20.3% ± 8.2% *vs* 3.2% ± 10.1% with standard medical therapy alone)^[102]. Considering that bi-level PAP with S-mode may aggravate central apnea through hyperventilation, this is not a good option for all HF patients with CSA. Conversely, studies using bi-level PAP with spontaneous and timed backup ventilation mode (*i.e.*, ST-mode) in chronic HF patients showed sufficient reduction in the AHI with one night of bi-level PAP and significant improvement in LV ejection fraction at 3 mo with bi-level PAP with ST-mode^[103–105]. In particular, in a study regarding the effects of bi-level PAP with ST-mode on the suppression of AHI and improvements in cardiac function in chronic HF patients with CSA that was not sufficiently suppressed by CPAP (*i.e.*, AHI ≥ 15, non-responders), CSA sufficiently decreased in response to bi-level PAP with ST-mode (AHI, from 54.4 ± 7.8 at baseline to 30.3 ± 11.7 on CPAP to 8.4 ± 4.7 on bi-level PAP with ST-mode)^[105]. Further, left ventricular ejection fraction (LVEF) and the plasma levels of B-type natriuretic peptide improved in chronic HF patients with CSA, even in patients deemed CPAP non-responders at 6 mo^[105]. Another PAP therapy that is more effective at suppressing AHI in chronic HF patients with CSA is ASV^[52]. Randomized and observational studies in which the effects of ASV on cardiac function were assessed showed that suppression of CSA *via* ASV reduced the levels of neurohumoral factors and improved LV systolic function and outcomes in chronic HF patients with CSA^[106–109]. Furthermore, in studies on the effects of ASV on suppression of AHI and improvements of cardiac function in CPAP non-responders, CSA was sufficiently decreased in response to ASV, and cardiac functions and neurohumoral state were improved at 3 mo^[110]. The effects of

ASV on long-term clinical outcomes in chronic HF patients with CSA will be clarified in an ongoing large-scale randomized controlled trial^[111,112].

Both OSA and CSA can be observed in patients with chronic HF, and ASV can suppress OSA by modifying the EPAP levels in addition to suppressing CSA. Thus, ASV, particularly ASV with auto-titrating EPAP, may be a therapeutic option for SDB without the need to distinguish between OSA and CSA. Three randomized controlled trials assessed the effects of ASV on cardiac function in chronic HF patients with coexisting OSA and CSA^[55,113,114]. These studies reported significant improvements in cardiac functions, especially reductions in neurohumoral factors. The effects of ASV for both types of SDB will be elucidated in an ongoing large-scale randomized controlled trial including chronic HF patients with either OSA or CSA^[115].

HF patients following acute decompensation

Although patients with ADHF are frequently treated with PAP therapy, whether HF patients following recovery from acute decompensation remains unclear. In HF patients following recovery from acute decompensation, the presence or absence of SDB may play key roles in determining whether PAP therapy should be considered. Although most previous data regarding SDB in HF and its treatment with PAP mentioned earlier involve HF patients in the chronic phase, it was recently reported that hospitalized HF patients following ADHF frequently develop SDB and that the presence of SDB during hospitalization following ADHF is a predictor of readmission and mortality^[116-118]. Thus, PAP therapy should be considered even for hospitalized HF patients, especially in the setting of symptomatic SDB. One study suggests a beneficial effect of in-hospital bi-level PAP (with S-mode) therapy for OSA on improvement of cardiac function following ADHF^[117]. An ongoing study may elucidate whether PAP therapy improve outcomes in these patients^[119]. However, there are no specific data regarding the effect of PAP therapy on hospitalized patients following ADHF who do not have SDB.

Chronic HF patients without SDB

Chronic HF patients even without SDB may also benefit from PAP therapy through its cardiac unloading effects. In fact, the short-term application of CPAP (*i.e.*, 5-10 cm H₂O) can increase cardiac output in stable HF patients with pulmonary congestion^[120,121]. This possibility has been further assessed in a subgroup analysis of a small randomized trial regarding the effects of CPAP on cardiac function and clinical outcomes in HF patients with and without CSA^[97]. In a subgroup analysis of patients without CSA, CPAP had no effect on either LVEF or the composite endpoint of mortality and cardiac transplantation rate. Bi-level PAP may be a better option for improving hemodynamics in HF patients with pulmonary congestion because net cardiac unloading effects during a respiration cycle may be greater in bi-level PAP than

in CPAP (refer to the section regarding “Bi-level positive airway pressure”)^[14,45]. Furthermore, based on data showing the acute beneficial effects of short-term ASV application on sympathetic nervous system activity^[33,34] and hemodynamics^[35], ASV may be a more promising therapeutic option for chronic HF patients without SDB. In fact, Koyama *et al.*^[122] reported that ASV was associated with better clinical outcomes, regardless of the presence or absence of moderate CSA (*i.e.*, AHI < 20 or ≥ 20). The possible benefits of ASV on cardiac function are being assessed in an ongoing randomized clinical trial in which HF patients with and without SDB are being randomized to either ASV treatment or medical therapy to assess the changes in LV ejection fraction at 6 mo^[123].

Most acute hemodynamic effects of PAP therapy are more prominent in HF patients with pulmonary congestion or increased LV filling pressure (*i.e.*, pulmonary capillary wedge pressure ≥ 12 mmHg)^[45,121]. Patients with HF are more sensitive to decreased afterload and are usually hypervolemic and are thus insensitive to decreased preload. However, preload reduction may play a more prominent role in HF patients without hypervolemia. Therefore, chronic HF patients with low filling pressure and those without hypervolemia should not be treated with PAP therapy or at least should be treated with caution.

HF with hypoventilation and hypercapnia

Among patients with HF, there is a subset of patients who have hypoventilation and hypercapnia acutely or chronically. In the acute phase, it was reported that 35 of 80 patients with acute cardiogenic pulmonary edema had hypercapnia that was not associated with a previous history of COPD^[124]. On the other hand, it was also reported that 25% of patients with ADHF had COPD^[125]. Thus, PAP therapy can be considered in such HF patients with hypoventilation and hypercapnia in the acute phase. In general, specifically designed ventilators for non-invasive PAP for acute or intensive care are used, although small home-care devices can also be used. In terms of modes, bi-level PAP or VPAS, both of which can provide sufficient minute ventilation or tidal volume to reduce PaCO₂, should be used. ASV may also be considered. However, because ASV is designed to keep PaCO₂ consistent in patients with hypocapnia and PaCO₂ oscillation, its effects for the reduction in PaCO₂ will be insufficient.

In the chronic phase, hypoventilation and daytime hypercapnia are observed in some elderly HF patients with COPD or in obese HF patients with OHS. Some patients with COPD can suffer from hypoventilation and daytime hypercapnia in association with individual variations in chemoreceptor sensitivity to CO₂ and inspiratory muscle strength^[126]. In addition, sleep-related hypoventilation and the initiation of long-term oxygen therapy can contribute to the development of hypoventilation and daytime hypercapnia in COPD patients. Mild physiologic hypoventilation during sleep, especially during rapid eye movement (REM) sleep, is exaggerated in patients with COPD. Hypoventilation and daytime hy-

percapnia can also be precipitated by supplemental oxygen therapy for hypoxia. Because both HF and COPD are more likely observed in elderly patients, the coexistence of HF and COPD has become more prevalent as the general population ages^[127]. Although the use of PAP therapy in COPD patients with chronic hypoventilation has not been established, the potential benefits of PAP therapy in these patients generally include improvement in daytime and nighttime arterial blood gas parameters, increase in sleep duration, improvements in quality-of-life^[128] and decreases in hospitalization rate^[129,130]. For patients with HF and COPD, PAP therapy can be used for cardiac unloading. Furthermore, it was reported that OSA occurs in 10% to 15% of patients who have COPD (*i.e.*, overlap syndrome)^[131]. In addition, HF patients frequently have OSA^[32]. Hypoventilation and hypercapnia in patients with HF and COPD can be attributed to coexisting OSA. Another means of PAP therapy in patients with HF and hypoventilation and hypercapnia is to suppress coexisting OSA.

In patients with chronic HF with hypoventilation and hypocapnia, the selection of the mode of PAP therapy is dependent on the volume of ventilation required to reduce the PaCO₂ levels. In patients who only require alleviation of coexisting OSA to reduce PaCO₂, CPAP can be used during sleep. If patients require pressure support to reduce PaCO₂, bi-level PAP can be used. If patients require a guarantee on delivered tidal volume or minute ventilation to reduce PaCO₂, VAPS can be used. ASV may also be considered. However, it should be noted that the effects of ASV for the reduction of PaCO₂ will be insufficient.

In obese HF patients with hypoventilation and hypercapnia, the coexistence of OHS [defined as obesity (body mass index > 30 kg/m²) and daytime hypoventilation with awake PaCO₂ > 45 mmHg in the absence of other causes of hypoventilation^[29]] should be considered. Patients with OHS frequently have multiple risk factors for cardiovascular disease in association with comorbid obesity. OHS can cause LV hypertrophy and diastolic dysfunction, and longstanding OHS may promote LV systolic dysfunction^[132]. In addition, OHS with severe hypoxia can cause pulmonary hypertension and subsequent right-sided HF. Therefore, OHS can induce the development and worsening of HF. Furthermore, approximately 90% of patients with OHS have OSA with and without REM sleep hypoventilation^[133]. In OHS, hypercapnia is due to increased work of breathing, OSA, respiratory muscle impairment, decreased central ventilatory drive, and decreased response to leptin. Obesity *per se* can increase the work of breathing through the increased efforts required to move the rib cage and the diaphragm and through decreased lung compliance. In addition to mild physiologic hypoventilation during sleep, OSA contributes to hypoventilation during each obstructive respiratory event, especially for REM sleep during which apneas and hypopneas become more severe in both frequency and

duration. Post-apnea (post-hypopnea) hyperpneas may not sufficiently compensate for hypoventilation to maintain eucapnia^[134] and reduced pH level and bicarbonate excretion at night as well as progressive elevation in the serum bicarbonate level and subsequent depression of ventilation during the day^[134,135]. Muscle impairment and decreased central ventilatory drive may play only a limited role in the pathogenesis of OHS^[131]. Although it was reported that alterations in leptin levels and leptin resistance can cause hypoventilation^[136], detailed mechanisms regarding these alternations in patients with OHS remain to be elucidated.

To treat HF patients with OHS, in addition to weight reduction, PAP should be considered to normalize ventilation and cardiac unloading. CPAP may be beneficial by preventing upper airway narrowing and hence improving alveolar hypoventilation, hypercapnia and oxygenation, and quality of life^[28,137,138] in some patients with OHS. However, some OHS patients still have significant nocturnal oxygen desaturation, even on CPAP^[139]. Providing pressure support with bi-level PAP should be considered for such patients and for those without OSA. Long-term bi-level PAP therapy improves hypercapnia, oxygenation, and increases lung volumes in patients with OHS^[140]. In an observational study, the use of bi-level PAP in OHS patients was associated with reduced mortality compared with patients who were not treated with bi-level PAP^[141]. Recent data suggest that VAPS may improve ventilation when compared with conventional bi-level PAP. However, the use of VAPS was associated with lower patient tolerance due to high pressure^[47,48]. Therefore, VAPS can be considered in patients who do not tolerate CPAP or bi-level PAP.

CONCLUSION

PAP is a non-invasive and non-pharmacological therapy for HF in the acute setting and is now globally used. In addition, in chronic HF patients with SDB, PAP therapy should be used to alleviate SDB and to improve short-term cardiovascular outcomes. Similarly, in HF patients with hypoventilation and hypercapnia in association with COPD and OHS, PAP therapy should be used to improve hypoventilation and hypercapnia. However, it remains to be elucidated whether PAP therapy can improve cardiovascular outcomes in patients following ADHF, in chronic HF patients without SDB, and in those with hypoventilation and hypercapnia. In particular, whether PAP therapy can alter long-term outcomes is of great interest. Therefore, further research regarding these topics is needed.

Nevertheless, cardiologists and other clinicians should understand the benefits of PAP therapy, including the improvements in the control of respiration and cardiac unloading, as well as the indications, contraindications and complications of this therapy, as discussed in this review.

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WJC 6th Anniversary Special Issues (5): Myocardial infarction

Magnetic resonance imaging and multi-detector computed tomography assessment of extracellular compartment in ischemic and non-ischemic myocardial pathologies

Maythem Saeed, Steven W Hetts, Robert Jablonowski, Mark W Wilson

Maythem Saeed, Steven W Hetts, Robert Jablonowski, Mark W Wilson, Department of Radiology and Biomedical Imaging, School of Medicine, University of California San Francisco, San Francisco, CA 94107-5705, United States

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Correspondence to: Maythem Saeed, Professor, Department of Radiology and Biomedical Imaging, School of Medicine, University of California San Francisco, 185 Berry Street, Suite 350, Campus Box 0946, San Francisco, CA 94107-5705, United States. maythem.saeed@ucsf.edu

Telephone: +1-415-5146221 Fax: +1-415-3539423

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Abstract

Myocardial pathologies are major causes of morbidity and mortality worldwide. Early detection of loss of cellular integrity and expansion in extracellular volume (ECV) in myocardium is critical to initiate effective treatment. The three compartments in healthy myocardium are: intravascular (approximately 10% of tissue volume), interstitium (approximately 15%) and intracellular (approximately 75%). Myocardial cells, fibroblasts and vascular endothelial/smooth muscle cells represent intracellular compartment and the main proteins in the interstitium are types I / III collagens. Microscopic studies have shown that expansion of ECV is an important feature of diffuse physiologic fibrosis (*e.g.*, aging and obesity) and pathologic fibrosis [heart failure, aortic valve disease, hypertrophic cardiomyopathy, myocarditis, dilated cardiomyopathy, amyloidosis, congenital heart disease, aortic stenosis, restrictive cardiomyopathy (hypereosinophilic and idiopathic types), arrhyth-

mogenic right ventricular dysplasia and hypertension]. This review addresses recent advances in measuring of ECV in ischemic and non-ischemic myocardial pathologies. Magnetic resonance imaging (MRI) has the ability to characterize tissue proton relaxation times (T1, T2, and T2*). Proton relaxation times reflect the physical and chemical environments of water protons in myocardium. Delayed contrast enhanced-MRI (DE-MRI) and multi-detector computed tomography (DE-MDCT) demonstrated hyper-enhanced infarct, hypo-enhanced microvascular obstruction zone and moderately enhanced peri-infarct zone, but are limited for visualizing diffuse fibrosis and patchy microinfarct despite the increase in ECV. ECV can be measured on equilibrium contrast enhanced MRI/MDCT and MRI longitudinal relaxation time mapping. Equilibrium contrast enhanced MRI/MDCT and MRI T1 mapping is currently used, but at a lower scale, as an alternative to invasive sub-endomyocardial biopsies to eliminate the need for anesthesia, coronary catheterization and possibility of tissue sampling error. Similar to delayed contrast enhancement, equilibrium contrast enhanced MRI/MDCT and T1 mapping is completely noninvasive and may play a specialized role in diagnosis of subclinical and other myocardial pathologies. DE-MRI and when T1-mapping demonstrated sub-epicardium, sub-endocardial and patchy mid-myocardial enhancement in myocarditis, Behcet's disease and sarcoidosis, respectively. Furthermore, recent studies showed that the combined technique of cine, T2-weighted and DE-MRI technique has high diagnostic accuracy for detecting myocarditis. When the tomographic techniques are coupled with myocardial perfusion and left ventricular function they can provide valuable information on the progression of myocardial pathologies and effectiveness of new therapies.

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Key words: Myocardial viability; Ischemic/non-ischemic

heart diseases; Magnetic resonance imaging; Multi-detector computed tomography; Cellular compartments; Contrast media

Core tip: This review addresses recent advances of measuring of extracellular volume (ECV) in ischemic and non-ischemic myocardial pathologies. The main approaches that are used for probing ECV are equilibrium contrast enhanced magnetic resonance imaging/multi-detector computed tomography and magnetic resonance imaging (MRI) longitudinal relaxation time mapping. These noninvasive techniques are currently used, but at a lower scale, as alternative to invasive endomyocardial biopsies to eliminate anesthesia, coronary catheterization and tissue sampling error. ECV measurements may aid in early detection of various myocardial pathologies. Delayed contrast enhanced-MRI (DE-MRI) and when T1-mapping demonstrated sub-epicardium, sub-endocardial and patchy mid-myocardial enhancement in myocarditis, Behcet's disease and sarcoidosis, respectively. Furthermore, recent studies showed that the combined technique of cine, T2-weighted and DE-MRI technique has high diagnostic accuracy for detecting myocarditis. When the tomographic techniques are coupled with myocardial perfusion and left ventricular function it can provide valuable information on the progression of myocardial pathologies and effectiveness of new therapies.

Saeed M, Hetts SW, Jablonowski R, Wilson MW. Magnetic resonance imaging and multi-detector computed tomography assessment of extracellular compartment in ischemic and non-ischemic myocardial pathologies. *World J Cardiol* 2014; 6(11): 1192-1208 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i11/1192.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i11.1192>

INTRODUCTION

Ischemic and non-ischemic cardiomyopathies have become a worldwide epidemic of the 21st century with increasing impact on healthcare systems. The 2012 European Society of Cardiology and 2013 American College of Cardiology Foundation/American Heart Association guidelines have set the stage for current therapy to reduce mortality and morbidity^[1,2]. Revascularization of coronary arteries in acute myocardial infarct (AMI) have become the treatment of choice and revascularization procedures have evolved significantly. Because X-ray coronary angiography-the clinically accepted reference standard for demonstrating coronary artery disease is invasive and provides information only on the anatomical status of coronary obstructive lesions several noninvasive methods have been developed to aid in the assessment of the functional status of myocardium, namely contraction and perfusion as well as microvascular and cellular integrity, including positron emission tomography and contrast-enhanced echocardiography. More recently, delayed contrast-enhanced (DE) magnetic resonance

imaging (MRI)^[3-13]. Extracellular MR contrast media identifies hyperenhanced infarct, hypoenhanced microvascular obstruction zone and a moderately enhanced peri-infarct zone in acute myocardial infarction^[5,6]. Delayed contrast enhanced-MRI (DE-MRI) has sensitivity of 99% for measuring AMI/scar infarct extent and 94% for measuring the transmural enhancement^[3,7,8]. Transmural enhancement was used to predict recovery of regional function in enhanced segments^[9]. A cutoff of 50% transmural enhancement was the threshold of recovery of regional function after intervention, where < 50% transmural enhancement predicted recovery in 53% of segments, while > 50% transmural enhancement was associated with negligible recovery (8% of segments)^[10]. Furthermore, < 25% transmural enhancement predicted residual viability in 82% of segments. DE-MRI has been clinically used to diagnose and specify different types of ischemic and non-ischemic cardiomyopathies based on the pattern and location of enhancement.

In ischemic cardiomyopathy the sub-endocardium is always enhanced on DE-MRI^[3,7,8], while in dilated cardiomyopathy, a patchy mid-myocardial pattern of enhancement is seen^[12]. Patients with mid-myocardial enhancement are at higher risk of sudden cardiac death and arrhythmias^[13]. Furthermore, patients with restrictive cardiomyopathy showed delayed myocardial enhancement over the entire sub-endocardial circumference^[14]. DE-MRI and T1-mapping demonstrated sub-epicardial, sub-endocardial and patchy mid-myocardial enhancement in myocarditis specific cardiomyopathies such as Behcet's disease and sarcoidosis, respectively. In Behcet's disease, enhancement of sub-endocardial fibrosis in the right ventricle is considered a feature of the disease. Vignaux^[15] observed delayed enhancement in sarcoidosis patients in specific locations [basal interventricular septum, lateral left ventricular (LV) wall] and distribution patterns (patchy or striate that do not involve the sub-endocardium) and in advanced cases of diffuse and focal pathologies. In non-ischemic dilated cardiomyopathy, Assomull *et al*^[13] showed that the presence of delayed myocardial enhancement was associated with a 3-fold increase of hospitalization for heart failure or cardiac death and a 5-fold increase of sudden cardiac death or ventricular arrhythmias. In hypertrophic cardiomyopathy, the extent of differentially enhanced myocardium assessed on DE-MRI was linked with progressive disease and markers of clinical risk for sudden death^[16].

Other MRI studies showed discordant results about the relationship between infarct enhancement and regional LV function. Beek *et al*^[17] reported that 25% of LV segments with transmural enhancement showed potential improvement in function at 13 wk. In another recent study, Dall'Armellina *et al*^[18] found that AMI does not necessarily equate with irreversible injury and severely underestimate salvaged myocardium on DE-MRI. Accordingly, new strategies have been developed to quantify diffuse myocardial fibrosis and small infarcted areas using equilibrium contrast enhanced magnetic resonance imaging/multi-detector computed tomography (MRI/MDCT)

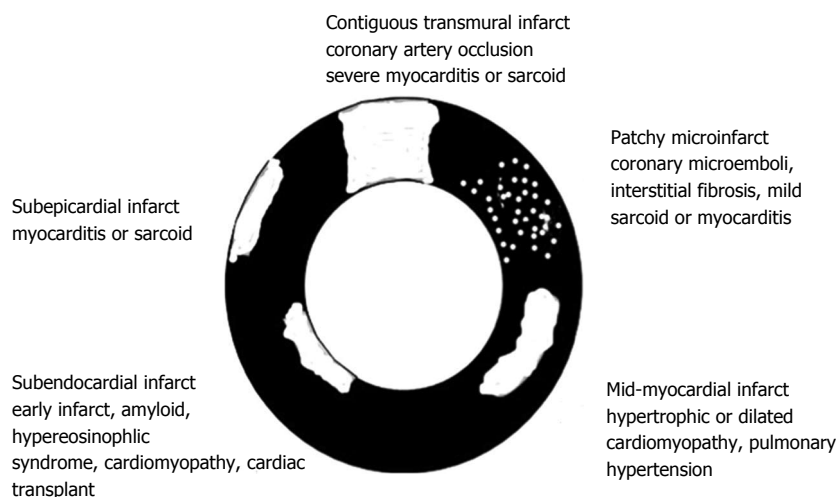


Figure 1 Schematic presentation of various types (patchy and contiguous) and locations (epicardium, midmyocardium and endocardium) of myocardial infarct in different cardiac diseases. In acute myocardial infarct > 30% of the patients have a hypoenhanced microvascular zone in the core of contiguous infarct. Reactive interstitial fibrosis is seen in hypertension, valvular, diabetic and genetic diseases as well as aging, while infiltrative interstitial fibrosis is evident in amyloidosis and Anderson-Fabry disease. The replacement of myocardium with scar tissue is seen in inflammatory disease, chronic ischemia/coronary occlusion (contiguous), chronic renal insufficiency (patchy) and genetic and toxic diseases.

and T1-mapping techniques^[19-27]. Lee *et al.*^[28] found that the extracellular volume (ECV) in healthy volunteers is stable between 8.5-23.5 min after gadolinium-based contrast media administration and in infarcted myocardium between 12-50 min^[29]. These methods overcome the question of the relationship between myocardial enhancement, function and diffuse fibrosis on delayed enhancement. They also allowed for the detection of greater collagen content in the extracellular compartment of myocardium in aging, failing heart, congenital heart, infiltrative heart, hypertension and hypertrophic cardiomyopathy pathologies than normal myocardium^[19,30-34].

Visualization of small infarcted areas, peri-infarct zone, patchy microinfarct and diffuse fibrosis remains difficult using existing DE-MRI and DE-MDCT because of low sensitivity, minor/vague alterations in tissue structure, nonspecific enhancement or overlapping with other confounding diseases. On the other hand, experimental studies have shown expansion of ECV in conditions where myocardial damage is invisible on MRI^[26,27]. A clinical MRI study found that the ECV of AMI is higher than the ECV in non-ischemic cardiomyopathies, suggesting that the damage is greater damage in the former. The study also showed that the location and pattern of enhancement differs between non-ischemic and ischemic cardiomyopathies^[35] (Figure 1).

MYOCARDIAL COMPARTMENTS

Microscopic studies revealed three fluid compartments in healthy myocardium, namely intravascular (approximately 10% of tissue volume), interstitial (approximately 15%) and intracellular (approximately 75%) compartment (Figure 2). It should be noted that the terms extracellular volume (ECV), volume of distribution, fibrosis index, and volume fraction of extravascular extracellular matrix share the same parameters for measuring the ECV by ad-

justing the contrast media partition coefficient with blood hematocrit^[26,27,36].

Intracellular water accounts for 79% of total water or about 380 mL/100 g of dry tissue and varies between individuals and species^[37]. The intracellular compartment includes myocardial cells, fibroblasts and vascular endothelial/smooth muscle cells. The main constituent proteins of the interstitial compartment are types I and III collagens. Water permeable membranes separate these compartments. Blood plasma and interstitial fluid exchange through pores and intercellular clefts in capillary endothelium.

The fluid in the interstitial compartment consists of a water solvent containing sugars, salts, fatty acids, amino acids, coenzymes, hormones, neurotransmitters and cellular waste products. The exchange of fluid and accompanying solutes between compartments is governed by hydrostatic and oncotic forces. These forces are typically balanced to maintain a constant fluid volume in the compartments. The molecular pathways that contribute to extracellular compartment remodeling post-MI, however, are multifactorial and related to; (1) the increase in osmotic colloidal pressure resulting from the leakage of plasma proteins^[38]; (2) the degradation of the extracellular matrix^[39]; and (3) heterogeneous or homogeneous loss of membrane integrity of myocardial cells. Disturbance in microvascular permeability causes extravasation of plasma macromolecules that subsequently leads to water imbalance and interstitial edema. Loss of the membrane integrity of myocardial cells further expands the extracellular compartment; and that is the basis for assessing viability and fibrosis (Figure 2). Expansion of ECV in ischemic and non-ischemic heart diseases is strongly associated with adverse outcomes^[40]. Expansion of ECV has been seen in myocarditis, hypertrophy, dilated cardiomyopathy, amyloidosis, congenital heart disease, aortic stenosis, restrictive cardiomyopathy, arrhythmogenic right

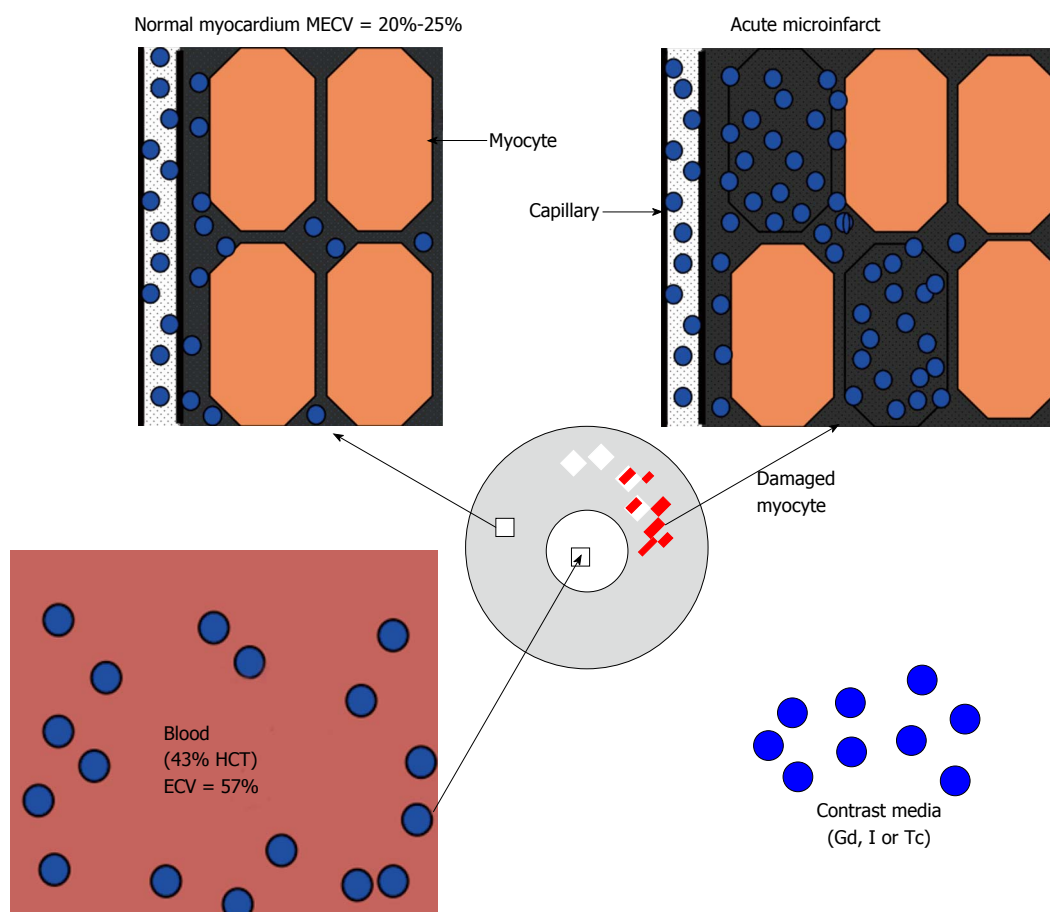


Figure 2 The three fluid compartments in healthy myocardium, namely intravascular (approximately 10% of tissue volume), interstitial (approximately 15%) and intracellular (approximately 75%) compartments. ECV: Extracellular volume; HCT: Hematocrit; Gd: Gadolinium; I: Iodine; Tc: Technetium; MECV: Myocardial extracellular volume.

ventricular dysplasia, hypertension and myocardial infarction (Figure 3).

Proton relaxation times (T_1 , T_2 , and T_2^*) reflect the composition of water protons in tissues. In 1992 several studies showed relationship between T_1 change and extracellular MR contrast media content in myocardium^[41-43]. Extracellular contrast media are rapidly distributed throughout the extracellular compartment in most tissues, but not in the brain, testis and retina. They are rapidly cleared from the circulation *via* the kidney. The quantity of contrast media distributed into a particular tissue is a function of physical extent of extracellular space and physiologic processes (blood flow, volume and diffusion) that distribute the agent into and remove it from the tissue. In myocardial infarct, investigators observed progressive alterations in structure and composition of the extracellular compartment^[44,45]. Interstitial edema in infarcted myocardium causes increase in longitudinal (T_1), transverse (T_2) and T_2^* relaxation times^[46] and administration of contrast media causes shortening^[19,30-32]. The decrease in the T_1 relaxation time is greater in infarcted than healthy myocardium, resulting in differential enhancement. T_1 assessment has also been used to measure macromolecular content, water binding and water content in tissues. The T_1 relaxation time is defined as the time when longitudinal proton magnetization

recovers approximately 63% of its equilibrium value. T_2^* relaxation time refers to decay of transverse magnetization caused by a combination of spin-spin relaxation and magnetic field inhomogeneity. The differential attenuation of infarct and viable myocardium on MDCT relies on X-ray absorption by iodine.

STRATEGIES FOR ESTIMATION OF ECV

The gold standard method for estimation of ECV in patients has been sub-endocardial biopsy. This method, however, has relatively high inherent risk, is limited to small regions and is prone to sampling site error^[47,48]. Visualization of large AMI and scar infarct on MRI and MDCT relies on the differences in signal intensity/attenuation between damaged and remote undamaged tissue to generate image contrast. It has been reported that undetected infarct account for at least 20% of all clinical cases of AMI and carry a prognosis as poor as detected ones^[49]. Furthermore, signal intensity on DE-MRI is displayed on an arbitrary scale and tissue signals or contrast media concentration cannot be quantified. Patchy microinfarct and diffuse fibrosis in non-ischemic myocardial cardiomyopathies necessitate alternative techniques beyond current DE-MRI or DE-MDCT. Fast MRI and MDCT image acquisition, T_1 sensitive sequences and

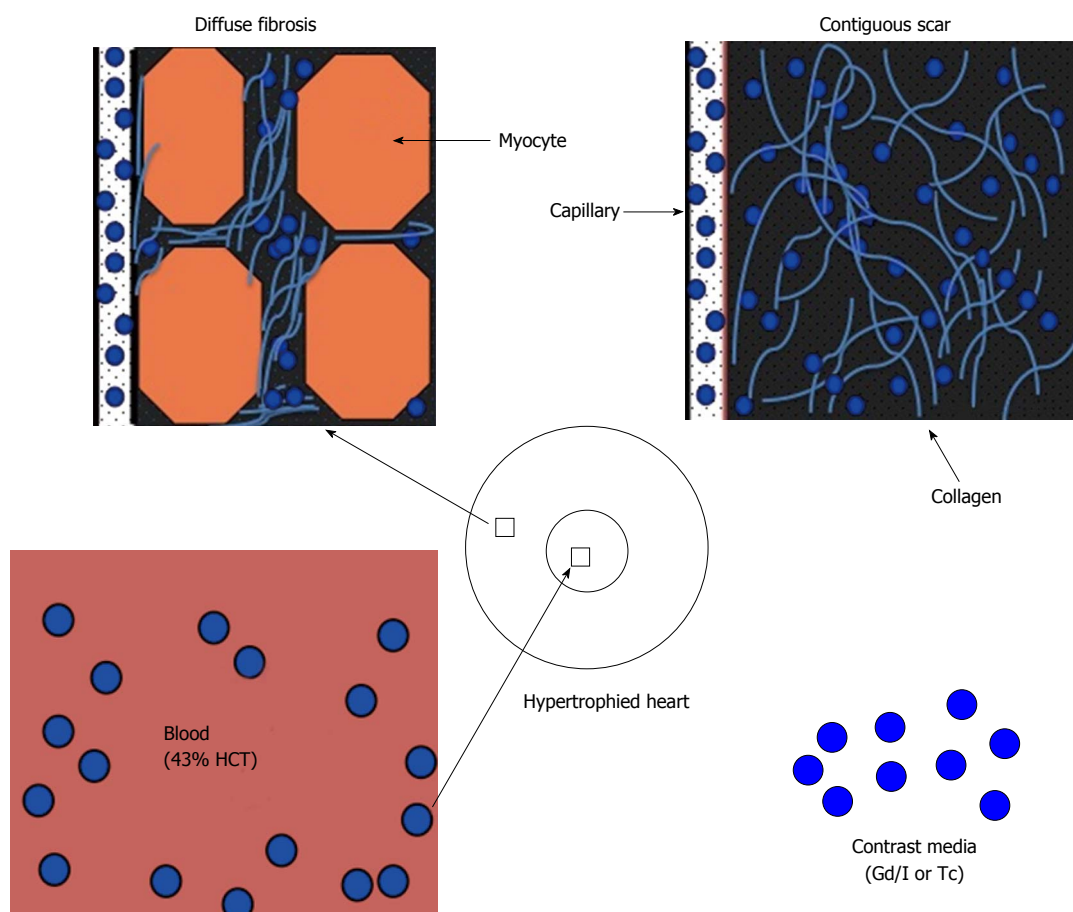


Figure 3 Schematic presentation of diffuse myocardial fibrosis in non-ischemic heart diseases (left) and contiguous chronic infarct (right) in ischemic heart disease. HCT: Hematocrit; Gd: Gadolinium; I: Iodine; Tc: Technetium.

contrast media allow the measurement of ECV. Look-Locker and echo planar MRI sequences as well as MDCT were used for non-invasive estimation of ECV. More recently, investigators have used MRI for T1 mapping and measuring ECV. The differences in regional T1 can be visualized as a grey-scale or color map^[50-53]. Investigators also found that equilibrium contrast and T1 mapping methods provide information, beyond what is visually evident on DE-MRI/DE-MDCT^[48,50]. These methods rely on three principles: (1) the measurement of global myocardial and blood T1 relaxation time/signal attenuation before contrast media administration; (2) a second measurement of T1 relaxation time/signal attenuation during contrast media equilibrium phase; and (3) a direct measurement of the blood contrast media volume of distribution. Extracellular inert gadolinium-based MR and iodinated computed tomography (CT) contrast media are crucial because they diffuse passively and rapidly between intravascular and extracellular compartments (Figure 4). Investigators have used longitudinal relaxation rate (1/longitudinal MR relaxation time; 1/T1) on MRI and myocardial signal attenuation on CT to quantify regional ECV^[22,41-43,54]. The calculation of ECV is based on the ratio of the difference in signal attenuation or 1/T1 before and after administration of contrast medium in myocardium divided by the difference in signal attenu-

ation or 1/T1 the blood pool. The increase in regional signal intensity on MRI and a decrease in attenuation on CT are attributed to the increase in ECV. Enhancement is expressed in Hounsfield or arbitrary units and employs tissue with lowest signal intensity as a reference for normality. The reason for using 1/T1 and not signal intensity on MRI is that signal intensity is not linearly correlated with contrast concentration. Unlike MR contrast media, signal attenuation after administration of CT contrast media is linearly correlated with contrast media concentration.

Our group was the first to find on MRI that the expansion in ECV is the mechanism for differential enhancement of infarct from healthy myocardium. We also demonstrated the peri-infarct zone^[26,27,55]. Later, Klein *et al*^[56] confirmed in patients with AMI that the partition coefficient is elevated in infarct compared to remote myocardium. Lee *et al*^[28] found in healthy volunteers that the ECV is $27\% \pm 1\%$ while Broberg *et al*^[33] found it is slightly lower ($22\% \pm 2\%$). Schelbert *et al*^[29] claimed that similar ECV values can be obtained by bolus ($21\% \pm 2\%$) and infusion ($25\% \pm 2\%$) approaches.

Recent studies have also shown that MDCT allows for assessment of myocardial viability and visualization of coronary stenosis^[57-59]. This imaging modality has been recently used for assessment of ECV in healthy volun-

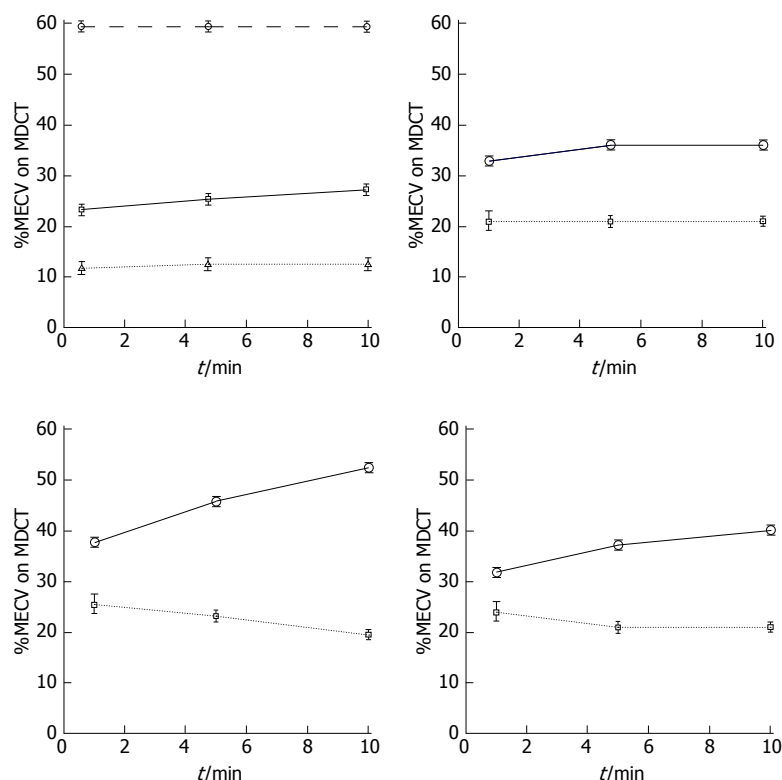


Figure 4 The top left plot shows the time course of equilibrium state of iodinated contrast media distribution in the extracellular volume of the blood (dashed line), healthy myocardium (solid line) and skeletal muscle (dotted line) over the course of 10 min using multi-detector computed tomography. The plots also demonstrate the remarkable difference in myocardial extracellular volume (MECV) in regions subjected to different insults. Differential increase in ECV was observed in ischemic myocardium after microembolization using 16 mm³ (top right), 32 mm³ (bottom left) or 90 min left anterior descending coronary artery occlusion/reperfusion (bottom right) compared with undamaged remote myocardium in all groups (dotted lines). MDCT: Multi-detector computed tomography.

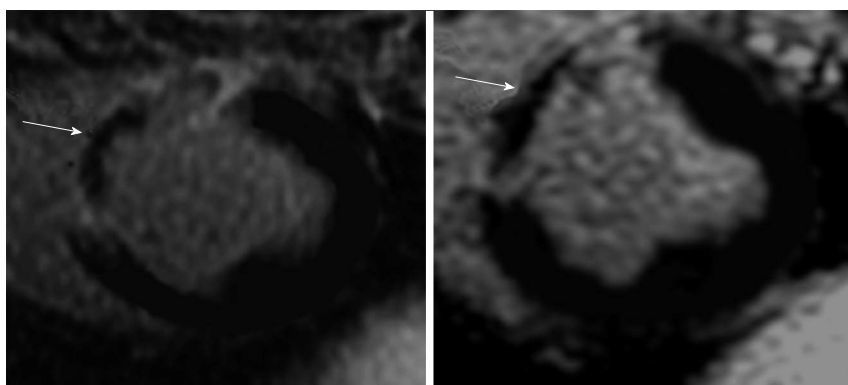


Figure 5 Delayed contrast enhanced magnetic resonance imaging of acute reperfused myocardial infarction (3 d) showing the hyperenhanced infarct and microvascular obstruction (arrows).

teers^[60] and infarcted swine hearts^[61]. Investigators found on MDCT that the ECV in healthy volunteers is between 23%-26%^[62,63]. We found in swine model of AMI that the ECV of iodinated contrast media is 24% in normal and 68% in infarcted myocardium (Table 1). Furthermore, the distribution volume of iodinated contrast medium was lower at the peri-infarct zone than infarct, suggesting that this zone contains admixture of viable and nonviable myocardial cells. In chronic infarct, the ECV in remote undamaged myocardium decreased to 18% as a result of compensatory hypertrophy (Table 1). Schelbert *et al*^[29] and Schmidt *et al*^[64] also observed extensive heterogeneity

in scar infarct, derangement in myocardial structure and accumulation of interstitial collagen that alters electrical activity and stiffens the myocardium. A clinical study showed that microvascular obstruction (MVO) occurs in > 30% of patients after ST segment elevation in myocardial infarction^[65]. The presence of MVO in the infarct is problematic in the assessment of ECV, because the rate of contrast wash-in and wash-out is severely reduced in MVO zone and the equilibrium state condition takes 18 min post contrast injection^[56]. Furthermore, the signal intensity of MVO zone is similar to remote undamaged myocardium (Figure 5).

Table 1 Multi-detector computed tomography quantification of extracellular volume in patchy microinfarct caused by microemboli, contiguous homogeneous infarct caused by left anterior descending coronary artery occlusion and remote undamaged myocardium

Intervention	Remote myocardium	Infarcted region
16 mm ³ and 3 d (AMI)	25 ± 4	33 ± 4 ^a
32 mm ³ and 3 d (AMI)	24 ± 2	40 ± 1 ^{a,c}
90 min LAD and 3 d (AMI)	24 ± 1	54 ± 4 ^{a,e}
90 min LAD and 5 wk (scar)	18 ± 2 ^b	68 ± 4 ^{a,g}

^a*P* < 0.05 *vs* remote myocardium; ^c*P* < 0.05 *vs* 16 mm³ microemboli volume; ^e*P* < 0.05 *vs* 32 mm³ microemboli volume; ^b*P* < 0.05 *vs* 90 min coronary artery occlusion/reperfusion at 3 d. AMI: Acute myocardial infarct.

MRI has inherent challenges that can be summarized as follows: (1) the presence of dental prostheses, orthopedic hardware or LV assist devices in the scanner; (2) slow acquisition time associated with high cost; (3) unsuitable for claustrophobic or uncooperative patients; (4) high technical and personnel requirements; and (5) MR contrast media provides a non-linear relationship between signal intensity and concentration^[66]. On the other hand, MDCT has the potential to accommodate a growing population of patients who are counter indicated for MRI. MDCT has different challenges such as: (1) presence of radiation exposure precludes serial assessment; (2) low contrast between infarct and normal myocardium; (3) requires post imaging reconstruction of images; and (4) lack of sequences analogous to MRI that provide circumferential/longitudinal LV strain data (such as tagging, phase contrast velocity encoded cine) or information on interstitial edema and hemorrhage (such as T2-weighted and T2*-weighted imaging).

SPECIFIC CARDIOMYOPATHY

Myocardial fibrosis (scar) can be related to either ischemic MI, non-ischemic cardiomyopathy, or the combination^[67]. For example, diffuse and contiguous fibrosis has been reported in heart failure, aortic valve disease and hypertrophic cardiomyopathy^[29,68-70], while solely diffuse fibrosis has been observed in myocarditis, hypertrophic/dilated cardiomyopathy, amyloidosis, congenital heart disease, aortic stenosis, restrictive cardiomyopathy (hyper eosinophilic and idiopathic types), arrhythmogenic right ventricular dysplasia and hypertension.

ISCHEMIC MI

T1 mapping has been widely used to assess non-ischemic cardiomyopathies. Recent studies show that this technique also has the potential to assess ischemic MI. Klein *et al.*^[71] determined ECV in 11 patients with heart failure and found that the ECV is greater in the infarcted region (54% ± 1%) than remote myocardium (29% ± 2%). Ugander *et al.*^[20] measured ECV in 126 patients with myocardial infarct and non-ischemic myocardial fibrosis and detected sub-clinical abnormalities in remote myocardium

using ECV measurements. They found that scar infarct has significantly higher ECV (51% ± 8%) than remote undamaged myocardium (27% ± 3%, *P* < 0.001, *n* = 36). In patients with non-ischemic cardiomyopathy, the ECV of atypically enhanced and remote myocardium were (37% ± 6% *vs* 26% ± 3%, *P* < 0.001, *n* = 30). They also observed in these patients that ECV of remote myocardium increased with the decrease of LV ejection fraction (*r* = -0.50, *P* = 0.02). A similar observation was reported in patients with heart failure^[48]. It has been shown that beta-blockers and angiotensin-converting enzyme inhibitors reduce diffuse myocardial fibrosis in patients with heart failure and hypertensive heart disease, respectively^[72,73], thus early measurement of ECV in suspected heart patients holds great promise for future clinical applications.

Coronary microembolization secondary to atherosclerotic plaque rupture occurs in spontaneously in patients with unstable angina/acute coronary syndromes^[74-76] and accidentally during coronary interventions^[77-83] with pathophysiological consequences, such as contractile dysfunction, perfusion-contraction mismatch, arrhythmias, myocardial ischemia and microinfarction^[84-87]. Clinical studies showed that revascularization of an occluded coronary artery, using PCI, coronary artery stents, or bypass grafting, causes visible and invisible patchy microinfarct^[88-91]. Both DE-MDCT and DE-MRI show promise in detecting patchy microinfarct caused by relatively large volumes of microemboli in a swine model (Figures 6 and 7)^[92-99], while equilibrium contrast enhanced MDCT provides a quantitative estimation of ECV as a function of microemboli volumes and duration of coronary artery occlusion (Figure 8). Histologic examination reveals dislodged microemboli in blood vessels surrounded with microinfarct (Figure 9). Small particles cause MVO, patchy microinfarct^[100], delayed infarct healing^[101], perfusion deficits and disturbances in ECG signal conductivity^[102,103]. ECV data derived from equilibrium contrast enhanced MDCT in a swine model are shown in Table 1.

NON-ISCHEMIC HEART DISEASES

Myocarditis

Myocarditis is the most frequent disease in patients with acute coronary syndrome and normal coronary arteries^[104]. Acute myocarditis is associated with systemic viral disease^[105,106]. At the early stage, there is myocardial injury/infarction, edema and regional/global LV dysfunction. On DE-MRI, myocardial injury is focal and located in the sub-epicardium and mid-myocardium (Figure 1). This method was also used for quantifying myocarditis^[107,108]. Furthermore, T2-weighted MRI sequence was also useful in detecting acute myocarditis for detecting interstitial edema, as an integral part of the inflammatory response, in acute myocarditis. This non-invasive method is useful for patients with acute chest pain, positive serum troponin and angiographically normal coronary arteries^[109,110]. Mahrholdt *et al.*^[110] speculated that the differential enhancement in the early phase is related to myocardial necrosis, but in the late phase to scar tissue. The sensitivity

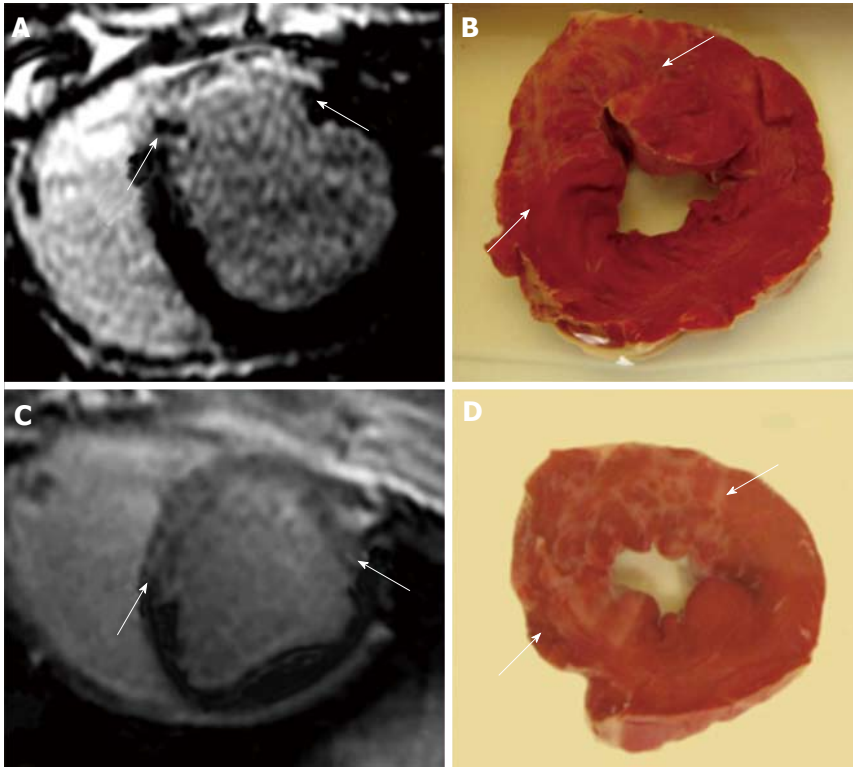


Figure 6 Delayed contrast enhanced magnetic resonance imaging (A and C) and histochemical triphenyltetrazolium chloride stain (B and D) show patchy microinfarct (arrows) 3 d after delivering 16 mm³ (A and C) and 32 mm³ (B and D) microemboli in the LAD coronary artery in a swine model.

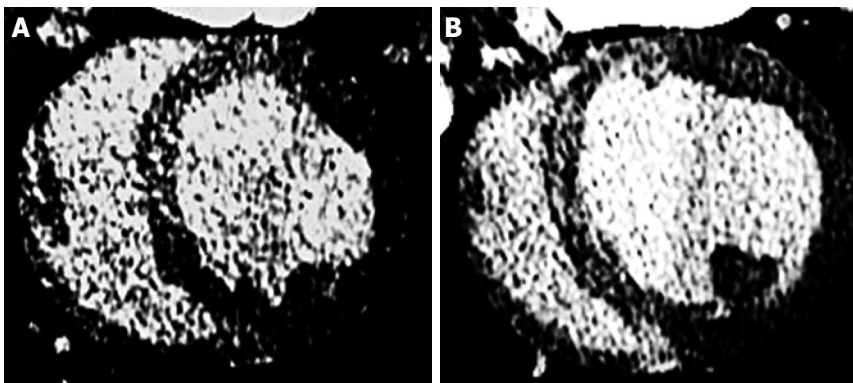


Figure 7 Delayed contrast enhanced multi-detector computed tomography 3 d after microembolization using 16 mm³ (A) and 32 mm³ (B) microemboli.

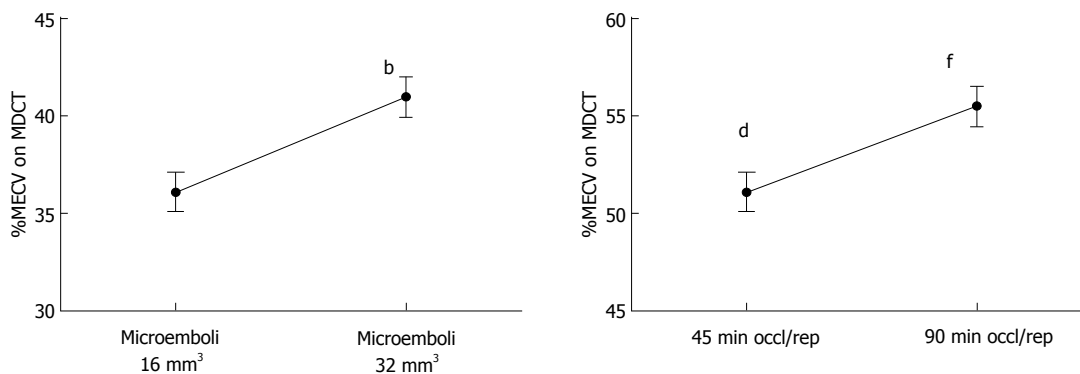


Figure 8 Gradient increase in myocardial extracellular volume as a function of microemboli volume (16 mm³ vs 32 mm³) and left anterior descending coronary artery occlusion time (45 min vs 90 min). ^b*P* < 0.01 vs 16 mm³, ^d*P* < 0.01 vs 32 mm³ and ^f*P* < 0.01 vs 45 min left anterior descending coronary artery occlusion/reperfusion. MECV: Myocardial extracellular volume; MDCT: Multi-detector computed tomography.

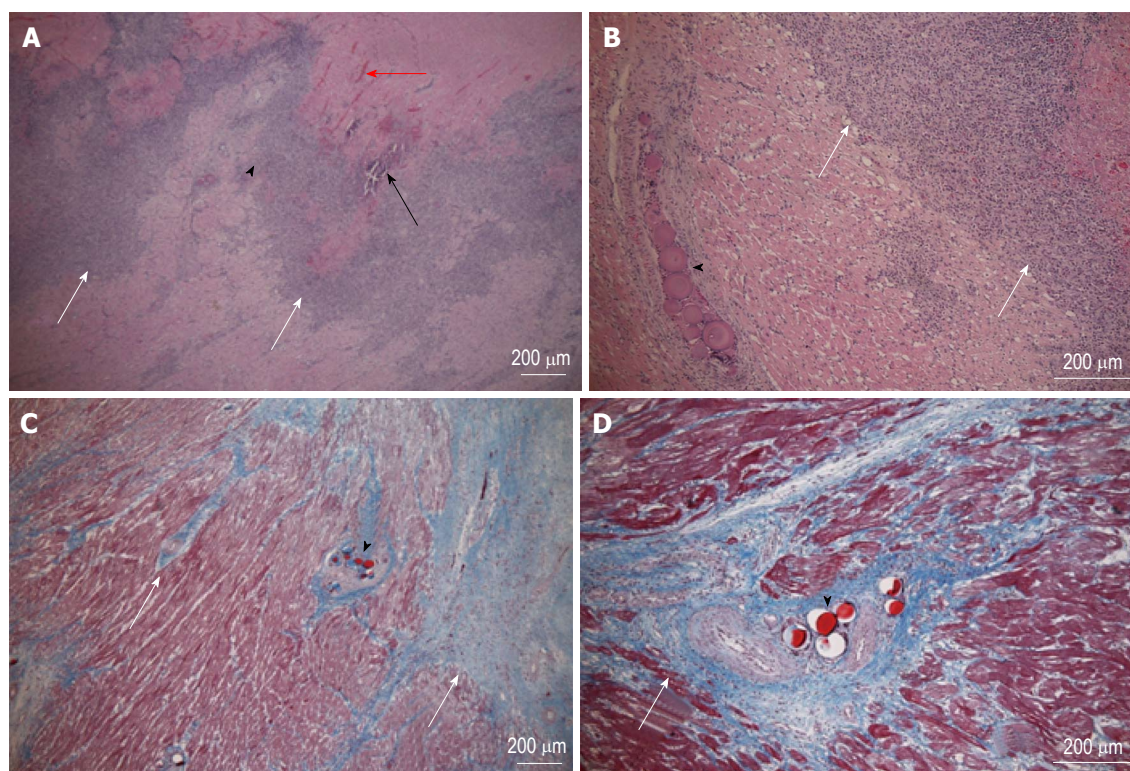


Figure 9 Acute (top row, hematoxylin and eosin stain) and chronic (bottom row, Masson trichrome stain) patchy microinfarct (white arrows) and microemboli (black arrowhead) distribution between viable myocardium at 3 d and 5 wk after embolization, respectively. Intramyocardial hemorrhage (red arrow) and calcium deposition (black arrow) are evident at 3 d on HE stain, but not at 5 wk. The magnifications are $40\times$ (A and C) and $100\times$ (B and D).

of DE-MRI in detecting myocarditis has been variable because of the different patterns (diffuse *vs* focal and acute *vs* chronic) of enhancement^[111-115]. More recently, Kellman *et al*^[52,116] found that ECV is significantly higher ($44\% \pm 6\%$) in myocarditic tissue compared with remote myocardium using T1 mapping.

Hypertrophic cardiomyopathy

LV hypertrophy is an independent risk factor for sudden death^[117,118]. Diffuse fibrosis is a common feature of hypertrophic cardiomyopathy and characterized by expansion of ECV and accumulation of interstitial collagen/fibrosis, which are hallmarks of pathologic remodeling^[119]. Because ventricular hypertrophy prevalence estimates in the general population are as high as 16% the public health implications are significant^[120].

DE-MRI in hypertrophic cardiomyopathy showed that both diffuse fibrosis and necrosis share mid-myocardium and sub-epicardium of the ventricular septum^[3,16,121,122] (Figure 1). Díez *et al*^[123] provided evidence for the role of fibrotic remodeling in hypertensive heart disease. Myocardial fibrosis in ventricular hypertrophy can impair the electrical coupling of myocardial cells by separating these cells with collagen and create a substrate of tissue heterogeneity from which re-entrant arrhythmias may arise^[124].

Recently, Shiozaki *et al*^[125] used MDCT to measure myocardial fibrosis in 26 patients with asymptomatic or mildly symptomatic hypertrophic cardiomyopathy. Myo-

cardial fibrosis was present in 25 of 26 patients (96%) with mean fibrosis mass of 21 ± 16 g, while patients with appropriate implantable cardioverter defibrillator shocks for ventricular tachycardia/fibrillation had significantly greater myocardial fibrosis than patients without (29 ± 19 g *vs* 14 ± 8 g; $P = 0.01$). For a myocardial fibrosis mass of at least 18 g, sensitivity and specificity for appropriate implantable cardioverter defibrillator firing were 73% and 71%, respectively^[125].

Clinical studies showed that myocardial fibrosis is reversible and treatable with timely intervention, therefore early detection and assessment is crucial. Investigators proposed that ECV measured on MRI may be useful in serially assessing the effects of therapies focused on proliferation of fibrosis in myocardium, such as the ACE-inhibitor (lisinopril)^[73] and the angiotensin II receptor antagonist losartan^[73,123]. These therapies have been shown to reduce the LV wall stiffness and severity of myocardial fibrosis (measured on biopsy) and concomitantly improve diastolic function.

DILATED CARDIOMYOPATHY

Dilated cardiomyopathy is an important cause of heart failure, sudden death and is the leading indication for cardiac transplantation in children and adults^[126]. MRI provides accurate assessment of ventricular chamber size, wall thickness, and systolic function. The pattern of DE-MRI can differentiate ischemic *vs* non-ischemic heart dis-

ease^[12]. For example, a sub-epicardial or mid-myocardial enhancement suggests non-ischemic cardiomyopathy. McCrohon *et al*^[12] reported that specific patterns of enhancement have been purported in dilated cardiomyopathy to indicate a particular genetic association; however, these findings are nonspecific. Other investigators showed that 59% of patients with dilated cardiomyopathy and normal coronary arteries have no delayed enhancement. The other 28% of patients had mid-myocardial enhancement that is consistent with a non-ischemic cause and few patients had delayed endocardial enhancement that is consistent with ischemic cause. Others found in patients with dilated cardiomyopathy that the extent of fibrosis has been associated with increased risk of intraventricular systolic dyssynchrony^[127].

CONGENITAL HEART DISEASE

Bandula *et al*^[54] developed an equilibrium CT protocol, using iohexol at 300 mgI/mL delivered as a bolus of 1 mg/kg and a rate of 3 mL/s, followed immediately by an infusion of 1.88 mL/kg per hour with CT imaging before and at 25 min after injection of bolus of contrast agent. The ECV within the myocardial septum in 23 patients with severe aortic stenosis was measured using both equilibrium CT and equilibrium MRI in patients. Biopsy samples of the myocardial septum were collected during valve replacement surgery and used for histologic quantification of extracellular fibrosis. They found that the mean percentage of histologic fibrosis was 18% and a significant correlation between both equilibrium MDCT derived and equilibrium MRI derived ECV and percentage of histologic fibrosis ($r = 0.71$, $P < 0.001$ and $r = 0.84$ ^[128], respectively). Equilibrium MDCT derived ECV was well correlated to equilibrium MRI derived ECV ($r = 0.73$). Broberg *et al*^[33] also found the fibrosis index was significantly elevated in patients with congenital heart disease compared with normal controls ($32\% \pm 5\%$ *vs* $25\% \pm 2\%$; $P = 0.001$), ECV values were highest in patients with a systemic right ventricle (L-transposition of the great arteries or D-transposition with prior atrial redirection surgery) and cyanosis ($35\% \pm 6\%$; $P < 0.001$ and $34\% \pm 6\%$; $P < 0.001$, respectively).

Ho *et al*^[129] were unable to visualize diffuse myocardial fibrosis in the setting of dilated cardiomyopathy using DE-MRI, but by T1 mapping technique, they found that fibrotic tissue has lower T1 relaxation time compared with healthy myocardium. Nacif *et al*^[22] successfully measured interstitial myocardial fibrosis in hypertrophied hearts using ECV measurement method. Others found that ECV is higher in subjects with hypertrophic cardiomyopathy ($36\% \pm 3\%$) than control volunteers ($27\% \pm 1\%$, $P < 0.001$). Furthermore, the ECV in hypertrophic hearts is heterogeneous and had substantially lower mean value than for scar infarct ($69\% \pm 9\%$, $P < 0.001$)^[116].

Neilan *et al*^[130] studied patients with hypertension and recurrent atrial fibrillation referred for pulmonary vein isolation underwent a contrast-enhanced MRI for measurement of ECV and were followed up prospectively

for a median of 18 mo. These patients had elevated LV volumes, LV mass, left atrial volumes, and increased ECV (patients with atrial fibrillation = $34\% \pm 3\%$; healthy control volunteers = $29\% \pm 3\%$; $P < 0.001$). They found positive associations between ECV and left atrial volume ($r = 0.46$, $P < 0.01$) and LV mass, but negative association between ECV and diastolic function ($r = -0.55$, $P < 0.001$). Furthermore, they demonstrated that each 10% increase in ECV is associated with a 29% increased risk of recurrent atrial fibrillation and concluded that ECV was the strongest predictor of the primary outcome of recurrent atrial fibrillation and the secondary composite outcome of recurrent atrial fibrillation, heart failure admission, and death.

AMYLOID

Amyloidosis refers to soluble proteins become insoluble, which are deposited in the extracellular compartment of various tissues, resulted in disrupting function^[131]. Amyloid heart disease is a systemic infiltrative disorder^[132]. It has been hypothesized that amyloidosis in myocardium is facilitated by hypoxia that results from capillary dysfunction. Endomyocardial biopsy has been considered to be the gold standard for demonstrating amyloid deposition in the heart. The most useful stain in the diagnosis of amyloid is Congo red, which, combined with polarized light, makes the amyloid proteins appear apple green on microscopy.

Noninvasive diagnosis of myocardial amyloidosis on MRI is difficult when this disease is accompanied with LV wall thickening related to hypertension^[133]. Amyloid heart reveals small infarcted areas on unenhanced T1 and T2 MRI^[134]. DE-MRI in myocardial amyloidosis is inherently challenging because amyloid infiltration within the extracellular compartment reduces the differences in contrast between LV chamber blood and myocardium such that the two regions may null simultaneously^[133,135]. On the other hand, other investigators found that the appearance of global and subendocardial enhancement on DE-MRI, is a unique characteristic of cardiac amyloid and correlates with prognosis^[136]. ECV was also measured in this disease using T1 mapping and gadolinium-based contrast media^[34,137]. It was found that the median ECV is significantly higher in infiltrative diseases (49% of tissue volume) compared with non-amyloid cardiomyopathy patients (33%) and volunteers (24%). The ECV strongly correlated with visually assessed segmental DE-MRI ($r = 0.80$) and LV mass index ($r = 0.69$), reflecting severity of myocardial infiltration. Sado *et al*^[35] reported that the ECV expansion is higher in systemic amyloidosis than in any other measured myocardial diseases, such as Anderson-Fabry disease, dilated cardiomyopathy, hypertrophic cardiomyopathy and hypertrophic cardiomyopathy, outside of the infarct zone. In another MRI study, Bandula *et al*^[54] studied 40 healthy volunteers and 67 patients with systemic amyloid light-chain amyloidosis of the upper abdomen using equilibrium MRI. They found that ECV was measured in the liver, spleen, and paravertebral mus-

cle. ECV was highest in the spleen (34%), followed by liver (29%) and muscle (9%). In patients with amyloidosis ECVs measured within the spleen (39%), liver (31%), and muscle (16%) were significantly higher than in healthy controls.

DIABETES

Type 2 diabetes mellitus promotes the expansion of ECV and increase vulnerability to a variety of clinical problems. Expansion of ECV is associated with mechanical dysfunction^[138-140], vasomotor dysfunction^[141], arrhythmia^[142] and mortality^[40,142]. Several studies support the notion that expansion of ECV contributes to adverse outcomes in diabetic patients. This notion is based on medications blocking the renin-angiotensin-aldosterone system ameliorate expansion of ECV^[143-145]. In a recent study, Wong *et al.*^[40] examined 1176 patients referred for MRI with and without diabetes. They found that diabetic patients ($n = 231$) had higher median ECV than non-diabetic patients ($n = 945$) (30.2% *vs* 28.1%, $P < 0.001$). More importantly, expansion of ECV measured by MRI appeared to be ameliorated with medications blocking the renin-angiotensin-aldosterone system. They concluded that diabetes is associated with increased ECV, which may be an important intermediate phenotype in diabetic individuals that is detectable by MRI-ECV and could be used as a biomarker to follow the effectiveness of diabetic treatment.

The ability to distinguish the sub-endocardial, mid-myocardium or sub-epicardial regions with true pathophysiology is a major limitation of ECV measurement. The presence of intra-myocardial fat also affects ECV measurements. The other major limitations of non-invasive MRI and MDCT in measuring ECV are the quality of the acquired images, presence of microvascular obstruction (may require continuous contrast infusion to reach equilibrium state of distribution)^[23,146] and the binding of contrast media to serum albumin that increases the relaxivity of some extracellular contrast media and decreases its diffusion^[147]. ECV measurement or T1 mapping are not intended to replace DE-MRI or DE-MDCT, which are excellent at depicting large infarction, but rather to be used in concert with cine and perfusion techniques. In tissues with a large intravascular compartment (such as the liver), the values of ECV may be overestimated by the equilibrium technique. The noise in the MDCT images stemming from beam-hardening artifacts originating from dense vertebral endplates is another limitation. Potential technical improvements in ECV measurements include faster processing of image data that reduce reliance on expert interpretation and increase the speed of image data processing.

Limitation

There are still multiple limitations in using MRI and MDCT for the assessment of ECV, such as the radiation dose, availability of experienced staff, extensive labor and the usage of contrast media. The side effects and cost of contrast media as well as costs of the scanner time

should be considered. Monitoring patients with severe heart failure or acute myocardial infarct inside the MR scanner is a difficult task.

FUTURE APPLICATIONS OF ECV MEASUREMENTS

New drugs, transplantation of different stem cells and local injection of genes have been recently introduced as potential therapies for infarct healing and myocardial regeneration. Chronological estimation of ECV may be useful for documenting the effectiveness of these therapies to promote myocardial viability. Recent clinical and experimental studies have shown that stem cells reduce infarct size and improve LV function in both AMI and scar^[148-151]. In a recent study Wong *et al.*^[40] described an association between measurements of ECV and clinical outcomes in a large patient cohort undergoing MRI. They analyzed 793 patients with known or suspected coronary artery disease, cardiomyopathy, or arrhythmias. They excluded patients with cardiac amyloidosis, infiltrative disease, hypertrophic cardiomyopathy and areas of delayed contrast enhancement consistent with classic pattern of myocardial infarction. They found that ECV ranged from 22%-26% in healthy volunteers, whereas it ranged from 21%-46% in the patients. Over a median follow-up period of 6 mo, 39 patients died, and 43 experienced a major adverse event (composite of death/cardiac transplant/LV assist device implantation). In multivariable modeling, ECV was associated with adverse cardiac events. For every 3% increase in ECV, there was a 50% increased probability of an adverse cardiac event. Furthermore, the potential of MRI/MDCT in guiding intramyocardial therapies and providing reliable and reproducible assessment of myocardial viability, perfusion and function has been recently reviewed^[152-154]. Further improvement in image resolution and processing of data would promise early detection and better pathophysiological understanding of diffuse fibrosis, myocardial infarct and help in timely intervention and therapy^[155].

In conclusion, since ischemic and non-ischemic myocardial diseases are characterized by an increase in the ECV, these pathologies can be characterized and may be differentiated on equilibrium contrast enhanced MRI/MDCT and T1 mapping. ECV data may provide a useful tool for diagnosis and treatment monitoring in ischemic and non-ischemic myocardial diseases (such as patchy microinfarct after percutaneous coronary intervention), compensatory hypertrophy, inflammation, heart failure and hypertrophic cardiomyopathy. The challenges lie in developing fast and sensitive imaging sequences, simple software for analysis, which will facilitate the ECV assessment approach into clinical routine practice.

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Blood glucose management in the patient undergoing cardiac surgery: A review

Pingle Reddy, Brian Duggar, John Butterworth

Pingle Reddy, Brian Duggar, John Butterworth, Department of Anesthesiology, Virginia Commonwealth University, Richmond, VA 232298-0695, United States

Author contributions: All authors conceived of the project, wrote and edited the manuscript, and are responsible for the content.

Correspondence to: John Butterworth, IV, MD, Professor and Chair of Anesthesiology, Department of Anesthesiology, Virginia Commonwealth University, PO Box 980695, Richmond, VA 232298-0695, United States. jbutterworth@mcvh-vcu.edu
Telephone: +1-804-8289160 Fax: +1-804-8288300

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Abstract

Both diabetes mellitus and hyperglycemia *per se* are associated with negative outcomes after cardiac surgery. In this article, we review these associations, the possible mechanisms that lead to adverse outcomes, and the epidemiology of diabetes focusing on those patients requiring cardiac surgery. We also examine outpatient and perioperative management of diabetes with the same focus. Finally, we discuss our own efforts to improve glycemic management of patients undergoing cardiac surgery at our institution, including keys to success, results of implementation, and patient safety concerns.

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Key words: Blood glucose management; Glycemic management; Cardiac surgery; Cardiothoracic surgery; Diabetes; Diabetes mellitus; Hyperglycemia; Perioperative

Core tip: There is a growing body of evidence that moderate glycemic control (*e.g.*, 120-180 mg/dL, 6.7-10.0 mmol/L) is an appropriate goal in cardiac surgery. Achieving this goal can be accomplished by

adopting a multidisciplinary approach, addressing the entire continuum of care, demanding a short project timeline, and identifying gaps in current management.

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INTRODUCTION

Diabetes is a common comorbidity in patients who require cardiovascular surgery. Worldwide, the total number of people with diabetes is projected to increase from 171 million in 2000 to 366 million in 2030^[1]. According to data from the National Diabetes Fact Sheet released in January of 2011, there are 25.8 million individuals with diabetes-which is more than 8% of the population-in the United States. In addition, based on fasting blood glucose and hemoglobin A1c levels, the authors of the National Diabetes Fact Sheet estimate that there are an additional 7 million people with undiagnosed diabetes and 79 million who are prediabetic and have a greatly increased risk of developing diabetes. The American Diabetic Association and the American College of Endocrinology classify prediabetics as those individuals with fasting blood glucose levels within the 100-125 mg/dL (5.5-6.9 mmol/L) range, while those with fasting blood glucose levels greater than 126 mg/dL (7.0 mmol/L) are considered to have diabetes mellitus^[2]. An estimate of the total cost of diagnosed diabetes in the United States was \$245 billion in 2012: \$176 billion for direct medical costs and \$69 billion in reduced productivity^[3]. Clearly, diabetes represents a major medical-economic problem in the developed world and the presence of diabetes complicates the management of the patient undergoing cardiovascular surgery. In this

review we will provide an overview of current data on best practices, techniques, and outcomes of glucose management in patients undergoing cardiovascular surgery. In addition, we will discuss how physicians can incorporate these findings into their own practices based on our own experiences and those of others.

HYPERGLYCEMIA AND ADVERSE OUTCOMES

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia as a result of a deficiency in insulin secretion, an increase in insulin resistance, or a combination of both. Type 1 (or “juvenile”) diabetes mellitus represents 5%-10% of all patients with the diagnosis of diabetes and is due to complete lack of insulin secretion by the pancreas. Type 2 diabetes mellitus, representing 90%-95% of all patients with the diagnosis of diabetes, is primarily due to insulin resistance resulting from multiple etiologies including genetic predisposition, unhealthy diet, lack of physical activity, and a characteristic central pattern of weight gain. Approximately 28% of diabetics will undergo coronary artery bypass grafting^[4,5].

Patients with diabetes have increased morbidity and mortality following coronary artery surgery^[6-8]. The incidence of stroke, renal failure, and sternal wound infections is greater in diabetic patients^[9-11]. Diabetics have a 44% greater risk for readmission (following hospital discharge after coronary artery surgery) for any cause and a 24% greater risk for readmission for heart-related issues than comparable nondiabetic patients who have undergone coronary artery surgery^[12,13].

DIABETES AND CARDIAC DISEASE

Hyperglycemia and insulin resistance lead to an alteration in free fatty acid metabolism, endothelial dysfunction, and resultant thrombogenesis^[14,15]. Hyperglycemia-induced endothelial dysfunction is the result of imbalance between nitric oxide bioavailability and the accumulation of reactive oxygen species, the latter triggered by activation of protein kinase C. Hyperglycemia also induces the generation of superoxide anion which inactivates nitric oxide to form peroxynitrite which induces substrate nitration^[16]. Diminished nitric oxide availability is a strong predictor of adverse nitric oxide outcomes^[17]. Protein kinase C also triggers the production of endothelin-1, which causes vasoconstriction, vascular inflammation and platelet aggregation^[18].

Hyperglycemia results in the production of advanced glycation products (AGE) and their cell surface receptor-RAGE. RAGE contributes to the inflammatory response by activating three key transcription factors: nuclear factor κ B, activated protein-1, and early growth response, all three of which are suppressed by insulin under normal conditions^[19-21]. Endothelial dysfunction also results from an increase in the synthesis of vasoconstrictors and prostanoids. Increased adiposity, a common feature in diabet-

ics, is strongly associated with increased concentrations of inflammatory markers and free fatty acids^[22]. Insulin resistance also promotes atherosclerosis by increasing triglycerides, apolipoprotein B, and low-density lipoproteins. In addition, concentrations of very low-density lipoproteins are generated in response to increased synthesis of apolipoprotein B^[23]. Coronary events in diabetics result from a prothrombotic state. Under normal circumstances, circulating concentrations of insulin inhibit platelet aggregation and thrombosis by inhibiting tissue factor and inhibiting production of plasminogen activator inhibitor-1 (PAI-1). In contrast, insulin resistance promotes increased synthesis of PAI-1 and fibrinogen as well as reduced production of tissue plasminogen activator. These factors collectively result in atherothrombosis^[24].

Key contributors to hyperglycemia-induced vascular damage include a newly identified class of RNAs termed micro RNAs (miRNAs) which regulate gene expression at the post-transcription level^[25,26]. Diabetics display a significant deregulation of the miRNAs involved in angiogenesis, vascular repair, and endothelial function^[27]. Ultimately, increased oxidative vascular stress causes thrombosis, impaired platelet function, and plaque rupture-all of which will result in reduced patency of grafts, reduced ischemic events, and a greater incidence of repeat revascularization in both coronary artery disease and diabetes^[28].

Hyperglycemia is associated with worse outcomes after acute coronary syndrome, acute myocardial infarction, or coronary artery surgery. Capes and coworkers performed a meta-analysis of 15 studies of patients without the diagnosis of diabetes who had glucose concentrations more than or equal to 110 mg/dL (6.1 mmol/L). Such patients had a 3.9 fold higher risk of death than patients without diabetes who had lower glucose concentrations. In patients without diabetes, glucose concentrations greater than 180 mg/dL (10 mmol/L) on admission were associated with increased risk of congestive heart failure or cardiogenic shock. Diabetic patients with glucose concentrations equal to or greater than greater than 180 mg/dL (10 mmol/L) had a moderately increased risk of death^[29]. Kosiborod *et al*^[30] analyzed admission glucose concentrations in 141680 elderly patients who were hospitalized for acute myocardial infarction. Twenty-six percent of these patients having glucose levels > 240 mg/dL (13.3 mmol/L) did not have the diagnosis of diabetes. Increased glucose concentrations were associated with a greater risk of 30-d mortality in patients without a previous diagnosis of diabetes (10%-39%) as compared to those patients with a diagnosis of diabetes (16%-24%)^[30]. In another review of 2127 patients with acute coronary syndrome, Foo *et al*^[31] showed a strong relationship between elevated glucose concentrations and an increased incidence of left ventricular failure and death. Meier *et al*^[32] analyzed data from 227 type 2 diabetics and 287 nondiabetics who were diagnosed with acute myocardial infarction. Hyperglycemia at the time of myocardial infarction was associated with shorter survival, larger infarct size, and an increased incidence of adverse outcomes in both diabetics and nondiabetics^[32].

Kubal *et al*^[11] analyzed the association of diabetes morbidity and mortality in 6033 patients undergoing isolated coronary artery bypass surgery. Insulin dependent diabetes was associated with an increased incidence of acute renal failure (adjusted OR = 4.5), deep sternal wound infection (adjusted OR = 2.96), and prolonged postoperative stay (adjusted OR = 1.60)^[11]. Gandhi *et al*^[33] analyzed glucose measurements and outcomes from 409 cardiac surgery patients and found that a 20 mg/dL (1.1 mmol/L) increase in mean intraoperative glucose concentration was associated with a 30% increase of an adverse event. Doenst *et al*^[34] in a retrospective review of 6280 cardiac surgery patients showed that a peak glucose of > 360 mg/dL (20.0 mmol/L) was associated with an increased likelihood of adverse events and mortality. Ascione *et al*^[35] in a retrospective review of 8727 cardiac surgery patients showed that glucose level > 200 mg/dL (11.1 mmol/L) at any time during the first 5 postoperative days was associated with an increased likelihood of in-hospital morbidity and mortality. Taken together, these studies suggest that hyperglycemia during acute coronary syndromes following cardiac surgery increases the likelihood of morbidity and mortality.

The Portland Diabetic Project as described in publications by Furnary *et al*^[36] provides strong evidence for an adverse linkage between hyperglycemia in diabetics undergoing cardiac surgery. This nonrandomized but prospective interventional trial involved 4864 diabetics. These investigators focused on the relationship between the use of a continuous insulin infusion and the incidence of perioperative mortality or deep sternal wound infections, and on length of hospital stay. Hyperglycemia was found to be an independent factor for increasing the likelihood of perioperative mortality. Those patients in which blood glucose remained < 150 mg/dL (8.3 mmol/L) were less likely to experience mortality (57% less likely) or deep sternal wound infections (66% less likely) as compared to diabetic patients whose blood glucose were “out of range”. Butterworth *et al*^[37] conducted a prospective, randomized trial of 381 nondiabetic patients undergoing cardiac surgery, where one group received a continuous insulin infusion attempting to maintain intraoperative blood glucose level less than a target level of 100 mg/dL (5.5 mmol/L) while the other group received no insulin. There was no difference in neurological or neuropsychological morbidity or in mortality between the two groups despite the insulin-receiving group having significantly lower intraoperative glucose levels^[37].

Hyperglycemia associates with adverse outcomes in patients with critical illness. Van den Berghe *et al*^[38] conducted a landmark study of 1548 ventilated patients. One group received insulin only if blood glucose exceeded 215 mg/dL (11.9 mmol/L) and had a target range of 180-200 mg/dL (10.0-11.1 mmol/L) while the other group received a continuous insulin infusion to maintain a blood glucose level between 80-110 mg/dL (4.4-6.1 mmol/L). Although intensive insulin therapy significantly reduced mortality in those patients requiring more than

five days in the intensive care unit (ICU), there was no difference in morbidity or mortality in those with ICU stays shorter than 3 d. Bhamidipati *et al*^[39] studied 4658 patients with known diabetes or perioperative hyperglycemia who were undergoing isolated coronary artery surgery. Patients in this study were stratified into a “tight group” (blood glucose concentrations < 126 mg/dL, 7.0 mmol/L), a “moderate group” (blood glucose concentrations 127-179 mg/dL, 7.0-9.9 mmol/L), and a “liberal group” (blood glucose concentrations > 180 mg/dL, 10.0 mmol/L). The moderate group had the lowest mortality 2.0% *vs* 2.9% in the tight group. Risk adjusted incidence of major complications was also less in the moderate control group suggesting that moderate control of hyperglycemia may be ideal for those diabetics undergoing isolated coronary artery surgery^[39].

OUTPATIENT DIABETES MANAGEMENT

Many patients who present for cardiac surgery have undiagnosed diabetes or metabolic syndrome. Such patients may have abnormally high blood glucose levels in the perioperative period and a significantly increased risk of adverse outcome. Of late, many institutions have formed multidisciplinary task forces involving the participation of representatives from pharmacy, anesthesiology, surgery, nursing, critical care, and endocrinology to provide better blood glucose control in patients undergoing and recovering from cardiac surgery. Some things are clear: diabetic care should be initiated in the preoperative period and not deferred until after the operation.

If possible, all cardiac surgical patients should have preoperative hemoglobin A1c (HbA1c) measurement. HbA1c levels reflect the adequacy of glycemic control in the 6-8 wk preceding the measurement. A HbA1c level of less than 7% indicates adequate glycemic control^[40]. Halkos *et al*^[41] found a significant association between HbA1c > 7.0% and a greater incidence of myocardial infarction, deep sternal wound infections, and mortality in patients undergoing coronary artery surgery. Some clinicians argue that elective coronary artery bypass surgery should be delayed when elevated HbA1c levels are detected to reduce the likelihood of perioperative complications. In a prospective study conducted by Lazar *et al*^[42], preoperative HbA1c levels were not predictive of 30 d morbidity, length of stay, or mortality following coronary artery surgery if glycemic control was achieved. However, this was a small study (*n* = 167) and a larger cohort would be needed to establish a definite conclusion regarding negative outcome associations with an elevated preoperative HbA1c measurement^[42].

The current recommendation from the Society of Thoracic Surgeons practice guideline is that oral hypoglycemics should be withheld for at least 24 h prior to surgery. Insulin dependent diabetics should not receive their nutritional insulins (regular, aspart, glulisine, lispro) once they have begun to fast after a meal the evening prior to surgery. neutral protamine hagedorn insulin (and other

intermediate or longer-acting insulins) should be reduced (on the day of surgery) from the usual dose to avoid intraoperative hypoglycemia. Many experienced clinicians will omit all subcutaneous insulin dosing on the day of surgery and substitute intravenous insulin infusion. Patients with a blood glucose concentration greater than 180 mg/dL (10.0 mmol/L) while awaiting elective surgery should receive a continuous insulin infusion to maintain their glucose concentration below 150 mg/dL (8.3 mmol/L). Once the patient is anesthetized we recommend that blood glucose be managed as if the patients were in the critical care unit (and we do not recommend “tight” control within the limits that would be used in ambulatory practice). Intraoperative blood glucose concentrations should be measured no less frequently than hourly. Patients with abnormal kidney function should be identified preoperatively since there is a greater incidence of perioperative hypoglycemia in these patients^[43,44].

HISTORY OF PERIOPERATIVE BLOOD GLUCOSE MANAGEMENT

Perioperative management of diabetes mellitus has greatly evolved over the past several decades^[45]. The scientific literature first recognized the importance of perioperative blood glucose control in the surgical patient in the early 1970s^[46]. At that point, the primary concern for anesthesiologists was avoiding ketoacidosis and acute hypoglycemia. Dr. Jurgen Steinke described the common techniques employed at the time in his 1970 review. He described obtaining urine specimens every four hours perioperatively and administering “sliding scale” subcutaneous insulin based on urine glucose measurements (*e.g.*, 15 U for a 4+ urine specimen, 10 U for a 3+ urine specimen, *etc.*). Dr. Steinke recognized the many flaws of this technique, including the assumption of normal renal function and that treatment was reserved for glucosuria and not for hyperglycemia *per se*. While the deleterious effects of chronic hyperglycemia on the cardiovascular system were recognized at that time, the morbidity associated with perioperative hyperglycemia in cardiac surgery patients had not yet been appreciated. Thus, no special considerations were made for patients undergoing cardiac surgery.

Throughout the 1970s, infused insulin became more widely used in caring for the patient with critical illness^[47-50]. Specifically, efforts to treat diabetic ketoacidosis with low-dose, continuous, infused insulin were met with considerable success^[49]. Therefore, investigators began studying the potential role of continuous insulin in diabetic patients undergoing surgery^[50]. In one report, Taitelman *et al.*^[50] described achieving better control of their diabetic surgical patients’ blood glucose with continuous insulin infusion (as compared to conventional subcutaneous “sliding scale”) as well as the unfortunate side effect of a more frequent incidence of hypoglycemia.

During the 1980s, a body of evidence was developed that linked poor glucose control in diabetics with poor

wound healing and increased rates of infection^[51,52]. The implications that this would have on diabetic patients undergoing surgery were clear, and by the late 1980s, algorithms for postoperative insulin infusions were widely available^[53]. In 1987, Watts *et al.*^[53] advocated a target plasma glucose range of 120 to 180 mg/dL (6.7 to 10.0 mmol/L) at a time when ideal blood glucose ranges were not well established. As a result of the lack of consensus on so many issues related to diabetic management, there was marked variation in accepted clinical practice.

In the 1990s, a multitude of outcomes-oriented clinical trials addressing diabetes in cardiothoracic surgery patients was reported^[12,54,55]. Now there was convincing evidence that diabetics were more likely to have wound infections, prolonged ICU length of stay, and mortality after cardiac surgery. Nevertheless, there remained no consensus on the ideal target range for blood glucose measurements. Consensus was reached (but only briefly) after Van den Berghe *et al.*^[38] 2001 prospective, randomized, controlled trial on intensive insulin therapy in 1548 critically ill patients. This study, which came to be known as the Leuven Surgical Trial, demonstrated reduced 12-mo mortality among critically ill patients when blood glucose levels were maintained in the 80-110 mg/dL (4.4-6.1 mmol/L) range as compared to 180-200 mg/dL (10.0-11.1 mmol/L). Mortality was 4.6% in the tight control group compared to 8.0% in the standard control group. The improved outcomes in the tight control group were attributed to fewer instances of multiple organ failure associated with sepsis. This led to an abrupt shift in how physicians cared for patients with critical illness. The publication of this study marked the beginning of the era of “tight control” in which standard care for critically ill patients, including those recovering from cardiothoracic surgery, mandated insulin infusion therapy.

Reports of several other important studies appeared during this time. For instance, the Portland Diabetic Project created and analyzed a large database of cardiac surgery patients ($n = 5510$) who underwent surgery between 1987 and 2005^[56]. These authors concluded that postoperative hyperglycemia rather than presence or absence of the diagnosis of diabetes was the true driver of increased mortality risk in the cardiac surgery patient. Van den Berghe *et al.*^[57,58] also continued to study the role of intensive insulin therapy in the critically ill during this time. In 2006, the group published two studies confirming the benefits of intensive insulin therapy in reducing the risk of morbidity and mortality in both medical and surgical ICU patients. These findings reinforced the prevailing notion that the tight control [*i.e.*, the 80-110 mg/dL (4.4-6.1 mmol/L) range that is used for tight control in ambulatory, nonsurgical practice] was also the ideal range for surgical patients in the perioperative period.

The era of tight glucose control in patients with critical illness came to an abrupt end with the publication of the NICE-SUGAR Study^[59]. These investigators were famously unable to reproduce the findings of the Leuven Surgical Trial. Here, 6104 patients were randomly assigned to either intensive control (target 81 to 108 mg/dL, 4.5

to 6.0 mmol/L) or standard control [target 180 mg/dL (10.0 mmol/L) or less]. Rather than experiencing the mortality benefit that Van den Berghe *et al.*^[38,57,58] found, the intensive control group actually experienced a greater incidence of all-cause mortality at 90 d after surgery (27.5% mortality in intensive group *vs* 24.9% in conventional group; 95%CI for the OR = 1.02-1.28; *P* = 0.02). These results caused physicians around the world to scale back the aggressive glycemic management protocols that were instituted during the era of tight control.

More recent studies were also unable to demonstrate a benefit of tight control^[60]. In 2011, Lazar *et al.*^[60] compared aggressive glycemic control (90-120 mg/dL, 5.0-6.7 mmol/L) against moderate control (120-180 mg/dL, 6.7-10.0 mmol/L) in 82 patients undergoing coronary artery bypass graft surgery. In this report, there was no difference in the incidence of adverse events between the groups (17 events in the moderate group compared to 15 events in the aggressive group, *P* = 0.91). Furthermore, hypoglycemic events were more frequent in the aggressive group (4 events in the moderate group compared to 30 events in the aggressive group, *P* < 0.0001). These results support the conclusions of NICE-SUGAR and suggest that moderate control (*e.g.*, 120-180 mg/dL, 6.7-10.0 mmol/L) may provide an appropriate balance between preventing adverse outcomes associated with perioperative hyperglycemia and avoiding dangerous hypoglycemic events.

ONGOING STUDIES

Diabetes and glucose control in the patient undergoing cardiac surgery remain subjects of intense research interest. For example, ongoing studies include “improving neurologic outcomes in diabetics undergoing cardiac surgery,” a clinical study ongoing at Wake Forest University (5R01HL089115). This study will address how genotype and phenotype interact to produce outcomes in patients with perioperative glucose intolerance. The hope is that with better classification of disease, management can be better tailored to meet the needs of individual patients. Ultimately, better perioperative management could lead to better perioperative glucose control and improved neurologic, neurobehavioral and other outcomes.

CURRENT GUIDELINES

After publication of the conflicting results from the Leuven Surgical Trial and the NICE-SUGAR Study, the ideal blood glucose range for patients with critical illness (and especially patients undergoing cardiac surgery) is once again ambiguous. Nevertheless, the 2009 Society of Thoracic Surgeons (STS) Guidelines are considered the current standard^[61]. The following Class I recommendations are included among these guidelines: (1) Patients taking insulin should not receive their nutritional insulin (lispro, aspart, glulisine, or regular) after receiving their dinner-time dose the evening prior to surgery (level of evidence = B); (2) Scheduled insulin therapy, using a combination

of long-acting and short-acting subcutaneous insulin or an insulin infusion, should be initiated to achieve glycemic control for in-hospital patients awaiting surgery (level of evidence = C); (3) All oral hypoglycemic agents and noninsulin diabetes medications should be withheld for 24 h prior to surgery (level of evidence = C); (4) All patients with diabetes undergoing cardiac surgical procedures should receive an insulin infusion in the operating room and for at least 24 h postoperatively to maintain serum glucose levels \leq 180 mg/dL (10.0 mmol/L) (level of evidence = B); (5) Glucose levels > 180 mg/dL (10.0 mmol/L) that occur in patients without diabetes only during cardiopulmonary bypass may be treated initially with a single or intermittent dose of intravenous (*iv*) insulin as long as levels remain \leq 180 mg/dL (10.0 mmol/L) thereafter. However, those patients with persistently elevated serum glucose (> 180 mg/dL, 10.0 mmol/L) after cardiopulmonary bypass should receive a continuous insulin drip, and an endocrinology consult should be obtained (level of evidence = B); (6) Patients (with or without diabetes) having persistently elevated serum glucose (> 180 mg/dL, 10.0 mmol/L) should receive *iv* insulin infusion to maintain serum glucose < 180 mg/dL (10.0 mmol/L) for the duration of their ICU care (level of evidence = A); and (7) Before intravenous insulin infusions are discontinued, patients should be transitioned to a subcutaneous insulin schedule using institutional protocols (level of evidence = B).

It is important to note that these guidelines were released before the publication of the NICE-SUGAR Study, so the information available at the time would not be considered complete today. The Guidelines Writing Group at the STS is currently working on updating these guidelines.

INSTITUTING A PERIOPERATIVE BLOOD GLUCOSE MANAGEMENT PROTOCOL

Instituting a new blood glucose management protocol can (and nearly always will) be a daunting task. While guidelines exist to define “guardrails” for insulin dosing and target glucose ranges, these guidelines provide little direction as to how best to implement the changes in practice and in culture that are so necessary to achieve those goals. Change management and the psychology of groups (particularly groups composed of “unequal” players) are beyond the scope of this manuscript^[62]. These topics are covered well in any number of management textbooks and monographs. Yet, experienced clinicians will recognize the key importance of group dynamics and negotiation skills to achieving success with a new clinical strategy. In other words, these issues cannot be ignored if the new strategy will succeed. Success cannot be achieved without “buy in” from physicians on the relevant clinical services. Nevertheless, nurses will drive the protocol in the ICU and on the hospital units; nurses must be involved in program development from the start. We have seen new clinical pathways fail due to the opposition of

a single, influential, antagonistic physician. Conversely, pathway success always requires an influential, trusted, and respected champion.

McDonnell *et al*^[40] published a primer in 2012 that provides some insight into the challenges that must be overcome when seeking to improve blood glucose management for cardiac surgery patients. At our institution, we encountered many of these temporary obstacles when we recently overhauled our perioperative glycemic management strategy in order to better comply with both STS Guidelines and Surgical Care Improvement Project (SCIP) requirements. We learned (or were reminded of) numerous lessons, a few of which are listed below:

Use a multidisciplinary approach

As previously noted, optimal glycemic control cannot be achieved through the efforts of a single physician or single medical discipline. We formed a process improvement team with representation from cardiac surgery, cardiac anesthesia, cardiac critical care, ICU nursing, endocrinology, clinical pharmacy, dietary, and the performance improvement department. Each discipline was responsible for a small subset of the project, and frequent meetings of the entire process improvement team allowed for ongoing progress updates and collaboration.

Address preoperative, intraoperative, and postoperative care at the same time

Glucose control in the preoperative, intraoperative and postoperative periods cannot be disentangled. Although it is tempting to address each stage of care in a piecemeal fashion, overall success requires the team to integrate these phases together. Having representation and periodic updates from those responsible for care at each point along the care continuum permits timely identification and remediation of persisting misconceptions or deviations from the plan.

Demand a relatively short project timeline (with well defined deadlines)

Process improvement projects can (and sometimes should) go on indefinitely. But, one will never see results if a strict timeline is not enforced. We recognize that the ideal approach to process improvement (since the time of Walter Shewhart and W. Edwards Deming) is a “plan-do-study-act” repetitive cycle, but also have seen that a team can get stuck on “plan” if the focus is on perfection rather than on improvement. The perfect course of action likely will never be determined; reaching a consensus can take an exorbitantly long time when discussion and debate are allowed to continue unchecked. We structured our discussions, allowing each discipline to take the lead on the facet of the project for which they were responsible. Our process improvement team met from May 2013 to August 2013.

Use flow charts to facilitate identification of “gaps”

Flow charts and process mapping were developed in industrial engineering to define precisely what is the desired

“product,” what are the individual steps in the process by which it is “made,” who is responsible for each step, and how can we measure our success at “manufacturing” this “product?” The process improvement team “mapped” glycemic management from patient admission to discharge during its first meetings. Each discipline described in detail the manner in which care was provided within their domain. Once the entire care continuum had been described, “gaps” in ideal care were identified. For example, representatives from anesthesiology identified that they had no standard blood glucose management protocol for the intraoperative period. Representatives of the dietary department pointed out that patients who had an order for a diabetic diet could still request sugar-sweetened soft drinks during the postoperative period. Once dozens of these potential gaps had been identified, the team determined which gaps fell under the purview of which disciplines, and then voted on which gaps should be prioritized for correction. This process allowed for the systematic identification and elimination in barriers to optimal glycemic control.

OUR SUCCESS IMPLEMENTING CHANGE

We monitored several outcome measures to evaluate the success of our newly instituted blood glucose management practices. Detailed explanations of these results are not the focus of this article, but broad trends are described here. Briefly, intraoperative blood glucose values fell within our target range 63% of the time for 35 consecutive patients who underwent cardiac surgery prior to the adoption of our new protocol. Thirty-eight consecutive patients undergoing cardiac surgery after to the institution of the protocol were similarly evaluated, and their blood glucose values fell within our target range 81% of the time ($P < 0.05$ using nonparametric tests).

Compliance with SCIP 4 measures for postoperative day one and two 6 am blood glucose values was also monitored [SCIP 4 requires postoperative day (POD) 1 and POD 2 blood glucose levels to be below 200 mg/dL]. Suboptimal performance on these measures during 2012 served as the impetus for the formation of our process improvement team. For that year, we achieved 90% compliance but lost considerable potential revenue in the value based purchasing program. For the 38 consecutive patients analyzed after the overhaul of our blood glucose management practices, we achieved 99% compliance on this SCIP 4 measure.

It is important to note that Institutional Review Board (ethics committee) approval including a waiver of consent was obtained in order to perform the chart review necessary to include these results here.

PATIENT SAFETY AND INSULIN INFUSION

The potential dangers of insulin therapy are well known to providers, and insulin infusion in the perioperative

setting is no exception. We experienced an example of the “Swiss cheese” model of error in which a series of unexpected, sequential actions were taken; omission of any one of these actions would have prevented a protocol deviation. The individual actions leading up to this patient safety “near miss” are listed here: (1) The infusion pump was programmed for a “basic” infusion rather than using preprogrammed “guardrails” for insulin infusions. The “guardrails” settings have built-in safeguards that alert the provider when excessive doses of a drug are entered. Using the basic infusion setting circumvents these safeguards; (2) An insulin infusion was intended to be programmed for 1.5 U/h but was erroneously programmed for 105 U/h; (3) Fortunately, this programming error occurred toward the end of the case, and the error was noticed immediately upon arrival in the ICU. As a consequence, we made several changes to our intraoperative protocol: We removed decimal points from the protocol such that infusion rates are rounded to the nearest unit rather than the nearest half-unit. This allows most infusion rates to be entered as a single digit, reducing the likelihood that a three-digit infusion rate will be set accidentally; (4) The safeguards built into the “guardrails” setting are now more explicitly stated within the protocol; and (5) Initiation of insulin infusion in the operating room now requires a second provider to double-check the correctness of the infusion (just as is done prior to any blood transfusion).

Even with the most stringent safeguards in place, one must keep in mind that every time an insulin infusion is started, there is an opportunity for a life-threatening error. Despite our best intentions, human error will not soon be eliminated from health care delivery^[63]. It is easy to point fingers and assign blame after a medical error, but it is far more productive to learn from mistakes and make whatever improvements are possible to the care pathways in which the error occurred.

CONCLUSION

The association between perioperative hyperglycemia and adverse outcomes after cardiac surgery is well established. It is less clear which clinical practices will optimize outcomes in these patients: efforts to tightly control blood glucose in cardiac surgery may lead to dangerous hypoglycemia. Van den Berghe *et al*^[38,57,58] showed benefits of aggressive insulin therapy to maintain tight control in the perioperative period, but later studies including NICE-SUGAR demonstrated that tight control was actually associated with worse clinical outcomes^[59]. As a result, tight control is no longer standard care for patients with critical illness. Even so, a consensus regarding the range of glucose concentrations for which clinicians should be aiming in these patients has remained elusive. There is a growing body of evidence that moderate control (e.g., 120–180 mg/dL, 6.7–10.0 mmol/L) is an appropriate goal. The Society of Thoracic Surgeons is expected to update their 2009 practice guidelines on perioperative glycemic management in the near future, so more formal

guidance will be available at that time.

Within a given institution, selecting a target glucose range is only the first step. Implementing a protocol to achieve that goal can be a challenging ordeal, and success is more often achieved when one addresses the entire continuum of care associated with blood sugar management. It is important to obtain buy-in from all those who will be involved in the care of patients undergoing cardiac surgery. Patient safety must be paramount throughout the design of a glycemic management protocol. Human error can never be completely eliminated. Wise clinicians will respond to patient safety events as opportunities for process improvement.

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Surgical management of moderate ischemic mitral valve regurgitation: Where do we stand?

Khalil Fattouch, Sebastiano Castrovinci, Giacomo Murana, Marco Moscarelli, Giuseppe Speziale

Khalil Fattouch, Department of Cardiovascular Surgery, GVM Care and Research, Maria Eleonora Hospital, 90100 Palermo, Italy

Sebastiano Castrovinci, Giacomo Murana, Department of Cardiovascular Surgery, University of Bologna, 40138 Bologna, Italy

Marco Moscarelli, Cardiothoracic Surgery Department, Hammersmith Hospital, Imperial College, London W12 0HS, United Kingdom

Giuseppe Speziale, Department of Cardiovascular Surgery, GVM Care and Research, Anthea Hospital, 70124 Bari, Italy

Author contributions: Fattouch K, Castrovinci S and Murana G contributed equally to this work and wrote the manuscript; Moscarelli M contributed to the editing of the manuscript; Speziale G contributed to the reviewing of the manuscript.

Correspondence to: Khalil Fattouch, MD, PhD, Chief of Cardiovascular Surgery Unit, Department of Cardiovascular Surgery, GVM Care and Research, Maria Eleonora Hospital, Viale Regione Siciliana 1571, 90100 Palermo, Italy. khalilfattouch@hotmail.com

Telephone: +39-91-6981111 Fax: +39-91-6761612

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surgical approach for the treatment of IMR remains debated. Some authors demonstrated that coronary artery bypass graft (CABG) alone is beneficial in patients with IMR. Conversely, in most patients, moderate IMR will persist or worsen after CABG alone which translate in higher long-term mortality as a function of residual mitral regurgitation severity. A probable reason for this unclear surgical management of functional MR is due to the contemporary suboptimal results of reparative techniques. The standard surgical treatment of chronic IMR is CABG associated with undersized annuloplasty using complete ring. Though, the recurrence of mitral regurgitation remains high (> 30%) because of continuous left ventricle remodeling. To get better long term results, in the last decade, several subvalvular procedures in adjunct to mitral anuloplasty have been developed. Among them, surgical papillary muscle relocation represents the most appreciated option capable to restore normal left ventricle geometry. In the next future new preoperative predictors of increased mitral regurgitation recurrence are certainly needed to find an individual time period of treatment in each patient with moderate IMR.

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Abstract

Ischemic mitral regurgitation (IMR) represents a common complication after myocardial infarction. The valve is anatomically normal and the incompetence is the result of papillary muscles displacement and annular dilatation, causing leaflets tethering. Functionally the leaflets present a restricted systolic motion due to tethering forces that displaces the coaptation surface toward the left ventricle apex. The patients present poor left ventricular function at the time of surgery and the severity of the mitral regurgitation increases the risk of mortality. Currently there is general agreement to treat surgically severe IMR nevertheless strong evidences for patient with moderate insufficiency remains poor and proper treatment debated. The most effective

Key words: Anatomy; Surgery; Cardiology; Valve; Mitral; Echocardiography

Core tip: Moderate ischemic mitral regurgitation should always be considered in patients undergoing other cardiac surgery. Restrictive anuloplasty alone fails as valid treatment because often associated with persistence and high recurrence rate of mitral regurgitation due to continuous ventricular remodeling. Probably more aggressive repair procedures addressing the subvalvular mitral apparatus would help to find more durable results for this complex disease. In the next future new preoperative predictors of increased MR recurrence are certainly needed to find an individual time period of treatment in each patients with moderate ischemic mi-

tral regurgitation.

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INTRODUCTION

Ischemic mitral regurgitation (IMR) occur in up to 40% of patients affected by myocardial infarction^[1]. IMR affects the myocardium rather than the valve itself and valve incompetence is the result of papillary muscles (PPMs) displacement, leaflet tethering, and annular dilatation. Functionally the leaflets present a restricted systolic motion due to tethering forces that displaces the coaptation surface toward the left ventricle (LV) apex^[2]. The patients present poor left ventricular function at the time of surgery and the severity of the mitral regurgitation increases the risk of mortality (lower among patients with mild IMR). Currently there is general agreement to treat severe IMR surgically, nevertheless evidences for patient with moderate insufficiency remain poor and proper treatment is debated.

ECHOCARDIOGRAPHIC CONSIDERATIONS

To define the severity of mitral regurgitation by Doppler echocardiography, the effective regurgitant orifice (ERO) area and the regurgitant volume (RV) are used. Organic MR is usually characterized by an ERO area > 0.4 cm² and RV > 60 mL/beat; these cut points are significantly lower for patients with functional MR (ERO area > 0.2 cm² and RV > 30 mL/beat, respectively)^[3,4].

MR severity in any individual patient should not be defined exclusively on the basis of few quantitative parameters but on an integrative evaluation that assess supplementary helpful findings, such as the pulmonary vein flow pattern, the size of left atrial and LV chambers. Lastly, since functional MR is an essentially dynamic lesion, the severity of regurgitation varies as a function of LV loading conditions and heart rhythm. For that reason, stress echocardiography is an important adjunct to the noninvasive evaluation of appropriate patients^[5].

OPEN CONTROVERSIAL ON SURGICAL MANAGEMENT

The most effective approach for the management of IMR remains discussed. Some authors demonstrated that coronary artery bypass graft (CABG) alone is beneficial in patients with IMR^[6,7]. Conversely, in most patients moderate IMR will persist or worsen after CABG

alone which translate in higher long-term mortality as a function of residual MR severity^[8]. A probable reason for this unclear surgical management of functional MR is due to the contemporary suboptimal results of reparative techniques^[9,10]. Undersized annuloplasty with complete ring, associated with CABG, presently is the most frequently performed surgical procedure to treat chronic IMR. However, recurrent MR can be expected in 1/3 of patients because of continued LV remodeling^[11,12]. There are many reviews about of adding subvalvular procedures to mitral anuloplasty to reduce the tenting forces and improve the long-term repair results^[13-15]. Recent experimental and clinical studies reported that displacement of the PPMs, due to LV remodeling, represents a key characteristic in the development of IMR; surgical papillary muscles relocation may represent a new precious instrument for surgeons^[15-18]. On the other hand, some authors reported very good results after mitral valve replacement^[19,20]. In a recent randomized trial^[21]. The Cardiothoracic Surgical Trials Network evaluate the relative risks and benefits of replacement versus repair, with or without CABG, in patients with severe IMR. As regard left ventricular reverse remodeling and 12-mo survival the authors observed no significant difference between mitral valve annuloplasty and replacement. However, in more than 30% of the patients in the repair group, a significant recurrent IMR developed. These data suggest a large potential benefit of valve repair if the effects of recurrent IMR can be limited. Therefore, the timing of valve repair in IMR needs to be assessed and patients with moderate regurgitation could benefit from early mitral surgery in morbidity though prolonged survival has to be demonstrated^[22].

FUTURE PERSPECTIVES

Currently there is general agreement to treat only severe IMR at the time of CABG. Conversely, according recent guidelines^[23,24], mitral valve repair should be considered for patients with chronic moderate secondary MR who are undergoing other cardiac surgery (class of recommendation II b and II a respectively for American heart association/American college of cardiology and ESC guidelines). Consensus opinions regarding best practices rely on studies that are retrospective, observational, and most often single centered^[25]. In 2009, the first trial on efficacy of adding mitral valves plasty to CABG for moderate IMR have been published by our group^[26]. We demonstrated that the effectiveness of adding mitral valve plasty to CABG was well demonstrated by the improvement of NYHA class and percentage of LVEF and by the decrease of MR, left ventricular end-diastolic and end-systolic diameters, left atrial size and pulmonary artery pressure. In the same direction the Randomized Ischemic Mitral Evaluation Trial support the addition of MVR to CABG in patients with moderate ischemic MR undergoing CABG^[27]. In this study, 73 patients referred for CABG with moderate IMR and an ejection fraction

> 30% were randomized to receive CABG plus mitral valve plasty (34 patients) or CABG only (39 patients). Moderate IMR was defined by an effective regurgitant orifice area of 0.20 to 0.39 cm², RV of 30 to 59 mL/beat, and vena contracta width of 0.30 to 0.69 cm. Mitral valve plasty was performed with insertion of a Carpentier-McCarthy-Adams ETlogix Ring (Edwards Lifesciences) in 85% of patients and a Carpentier-Edwards Physio Ring (Edwards Lifesciences) in 15% of patients. Mean mitral leaflet coaptation length was 7.1 ± 1.2 mm, and technical success was defined as no or trivial MR intra-operatively. The authors demonstrated that the addition of mitral valve repair by anuloplasty to CABG reduced MR severity, LV volumes, and BNP levels, with an improvement in functional capacity and symptoms at 1 year. Longer-term follow-up of MR severity in both treatment groups would be of interest because LV reverse remodeling continue for up to 2 years after coronary artery revascularization, and it is possible that patients in the CABG-only group may demonstrate greater reverse remodeling with time. Unfortunately, in both trials^[26,27] there are no data on the use of cardiac resynchronization therapy when appropriate, strongly encouraged in guidelines. Another randomized, controlled multicenter trial in patients with moderate IMR is ongoing (ClinicalTrials.gov NCT00806988) designed to assess the effect of mitral valve repair added to CABG surgery on the combined end point of survival and re-hospitalization for heart failure in patients with moderate IMR followed for 5 years^[28]. Moreover, the Cardiothoracic Surgery Network will shortly complete enrollment of 300 patients in a companion study of CABG plus mitral valve repair versus CABG alone in patients with moderate IMR^[29]. Results from these trials will further elucidate the optimal treatment algorithm for patients with IMR; however, discrepancies in trial design, echocardiographic inclusion/exclusion criteria, and surgical technique suggest a continued role for large observational studies to facilitate a valid management of these patients. A key point could be to improve patient selection to identify more precisely which individuals will benefit from surgical intervention. In particular, stress tests could be very helpful to determine the precise time of intervention in this clinical setting. In particular, recent research efforts concentrated on exercise echocardiography^[5,30]. Hung *et al.*^[11], for example, demonstrated as CABG alone left more patients with heart failure symptoms at rest and during exercise. This diagnostic tool should always be considered pre-operatively because induced dyspnea, increased in MR severity and systolic pulmonary artery pressure are often disguised in patients with moderate IMR at rest. Only a proper preoperative evaluation would not leave patients un-correctly treated. Therefore, this new clinical strategy would maximize the beneficial effects of repair and neutralize the effects of recurrent IMR. In the next future, the research for preoperative predictors of increasing MR recurrence and for alternative reparative approaches are probably the two key points to find an individual treat-

ment in each patients with this complex post-ischemic complication.

CONCLUSION

Moderate IMR should always be considered in patients undergoing other cardiac surgery. Restrictive anuloplasty alone fails as valid treatment because often associated with persistence and high recurrence rate of MR due to continuous ventricular remodeling. Probably more aggressive repair procedures addressing the subvalvular mitral apparatus would help to find more durable results for this complex disease. In the next future new preoperative predictors of increased MR recurrence are certainly needed to find an individual time period of treatment in each patient with moderate IMR.

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Primary angioplasty for infarction due to isolated right ventricular artery occlusion

Anwar A Chahal, Min-Young Kim, Alexander N Borg, Yahya Al-Najjar

Anwar A Chahal, Min-Young Kim, Alexander N Borg, Yahya Al-Najjar, Department of Cardiology, Blackpool Victoria Hospital, Blackpool FY3 8LP, United Kingdom

Author contributions: Borg AN and Al-Najjar Y were involved in direct patient care; Chahal AA and Borg AN wrote the manuscript, including preparation of figures; Kim MY and Al-Najjar Y edited and referenced the manuscript; Kim MY acted as the corresponding author for publication process.

Correspondence to: Dr. Yahya Al-Najjar, Department of Cardiology, Blackpool Victoria Hospital, Whinney Heys Road, Blackpool FY3 8LP, United Kingdom. ys.najjar@gmail.com
Telephone: +44-12-53300000

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Abstract

We report an unusual case of an isolated right ventricular infarction with haemodynamic compromise caused by spontaneous isolated proximal occlusion of the right ventricular branch of the right coronary artery (RCA), successfully treated by balloon angioplasty. A 58-year-old gentleman presented with epigastric pain radiating into both arms. Electrocardiograph with right ventricular leads confirmed ST elevation in V4R and a diagnosis of isolated right ventricular infarction was made. Urgent primary percutaneous intervention was performed which revealed occlusion of the right ventricular branch of the RCA. During the procedure, the patient's blood pressure dropped to 80/40 mmHg, and echocardiography showed impaired right ventricular systolic function. Despite aggressive fluid resuscitation, the patient remained hypotensive, continued to have chest pain and persistent electrocardiograph changes, and hence balloon angioplasty was performed on the proximal right ventricular branch which restored flow to the vessel and revealed a severe ostial stenosis. This was treated with further balloon angioplasty which restored TIMI 3 flow with resolution of patient's symptoms. Repeat echocardiography showed complete resolution of the

ST-elevation in leads V4R and V5R and partial resolution in V1. Subsequent dobutamine-stress echocardiography at 4 wk showed good left and right ventricular contractions. The patient was discharged after a 3-d in-patient stay without any complications.

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Key words: Right ventricular infarction; Right ventricular branch occlusion; Angioplasty; Myocardial infarction; Rare

Core tip: We describe an unusual case of an isolated right ventricular infarction caused by spontaneous proximal occlusion in the right ventricular branch of the right coronary artery (RCA), successfully treated by balloon angioplasty. Isolated right ventricular infarction (IRVI) is a rare presentation of occlusion of the right ventricular branch of the RCA. Most incidences of IRVI in the literature have been reported as complications to percutaneous intervention to the RCA.

Chahal AA, Kim MY, Borg AN, Al-Najjar Y. Primary angioplasty for infarction due to isolated right ventricular artery occlusion. *World J Cardiol* 2014; 6(11): 1223-1226 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i11/1223.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i11.1223>

INTRODUCTION

Isolated right ventricular infarction (IRVI) is a rare presentation of occlusion of the right ventricular (RV) branch of the right coronary artery (RCA). Most incidences of IRVI in the literature have been reported as complications to percutaneous intervention to the RCA. There have been only two other reports describing spontaneous RV branch occlusion leading to IRVI. We describe an unusual case of IRVI caused by a spontane-

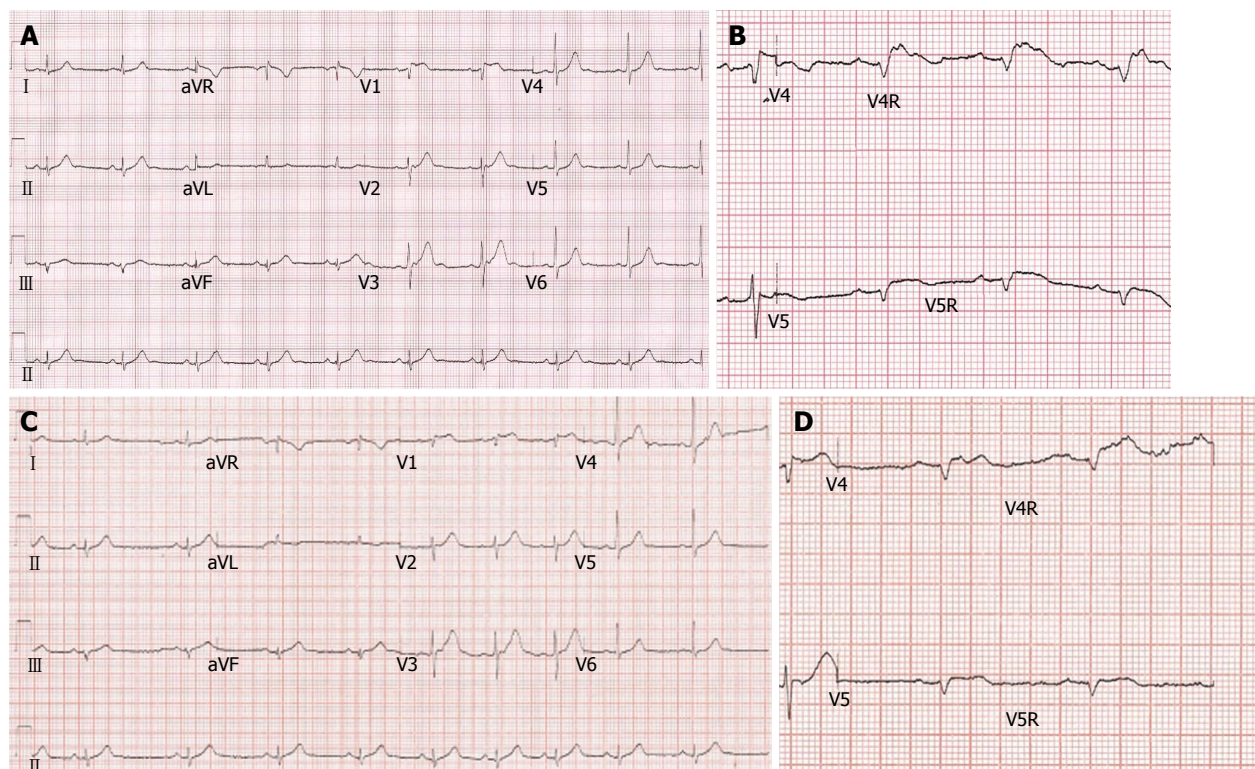


Figure 1 Electrocardiography. A: Standard 12-lead ECG at time of presentation showing ST elevation in lead V1; B: Right-sided ECG leads showing ST elevation in V4R and V5R; C: 12-lead ECG post-primary percutaneous intervention showing partial resolution of ST elevation in V1; D: Right-sided ECG post-percutaneous intervention showing resolution of ST elevation in V4R and V5R. ECG: Electrocardiography.

ous proximal occlusion in the RV branch of the RCA, successfully treated by balloon angioplasty.

CASE REPORT

A 58-year-old gentleman with no significant past medical history presented to the emergency department with sudden onset epigastric pain radiating to both arms. Initial electrocardiography (ECG) showed 3 mV ST elevation in lead V1 (Figure 1A). A subsequent ECG with right ventricular leads V4R and V5R confirmed ST elevation (Figure 1B) and a diagnosis of IRVI was made. Chest X-ray and clinical examination were unremarkable. The patient was given aspirin 300 mg, ticagrelor 180 mg, atorvastatin 80 mg and was transferred to our hospital for an urgent primary percutaneous intervention (PCI). The right radial artery was accessed using a 6F sheath (Glidesheath, Terumo) and angiography performed using a 5F Judkins left 3.5 diagnostic catheter and a Judkins right 4 guide catheter. The left main stem was free of significant atheroma. The left anterior descending artery had a 30% focal atherosclerotic plaque in the proximal vessel with a further mild mid-vessel stenosis. The circumflex artery and obtuse marginal branches did not have any significant atheromatous changes. The RCA was noted to be dominant and of moderate calibre. There was 40%-50% stenosis of the mid RCA and a moderate-severe ostial stenosis of the posterior descending artery (PDA) with TIMI 3 flow through the main vessel. Careful scrutiny

of the images showed absence of the RV branch of the RCA (Figure 2A). At this point, the patient's blood pressure had dropped to 80/40 mmHg. Despite aggressive fluid resuscitation, there was little improvement in the BP and the patient had ongoing symptoms of chest pain with persistent ECG changes. Therefore, with Bivalirudin cover, the RV branch lesion was crossed with a hydrophilic Runthrough wire (Terumo) with an additional balance middle weight (Abbott Vascular) "buddy" wire to the distal RCA (Figure 3). This was followed by balloon angioplasty of the proximal RV branch with a 1.2 mm × 10 mm MINI TREK (Abbott Vascular) compliant balloon (Figure 4), which restored flow to the vessel and revealed a severe ostial stenosis. This was treated with further balloon angioplasty using a 2 mm × 6 mm non-compliant Quantum Apex (Boston Scientific) balloon. The final angiographic result showed restoration of TIMI 3 flow (Figure 2B) with resolution of chest pain. A bolus of Bivalirudin (a direct thrombin inhibitor) was administered, followed by an infusion.

The patient received routine post-MI care on the coronary care unit. The peak 12-h Troponin-I was 16628 ng/L from the initial 1115 ng/L (normal 0-59 ng) and creatine kinase was 632 IU/L (normal 25-175 IU/L). A transthoracic echocardiogram showed a normal sized left ventricle (LV) with preserved systolic function, but the RV had reduced radial systolic contraction of the basal and mid-segments of the free wall. The patient was discharged after an uncomplicated 3 d in-patient stay.

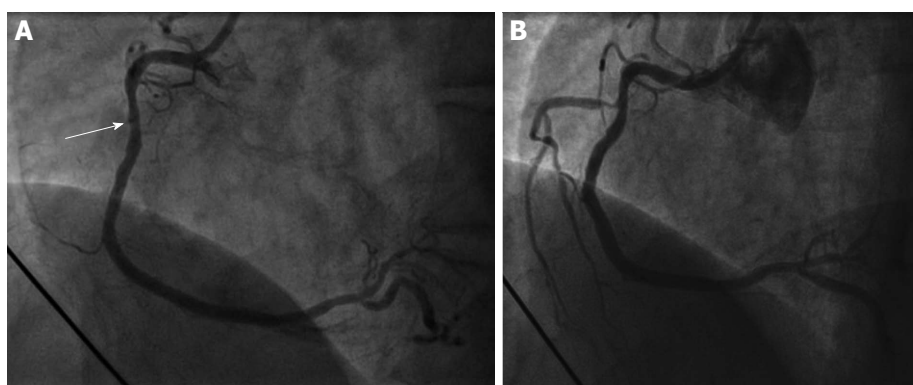


Figure 2 Balloon angioplasty for right coronary. A: Cranial view of right coronary artery showing occluded right ventricular branch (white arrow); B: Restoration of TIMI 3 flow in right ventricular branch after balloon angioplasty.



Figure 3 Right anterior oblique view of balance middle weight wire crossing the proximal lesion in the right ventricular branch. There is a "buddy" wire in the main right coronary artery vessel.

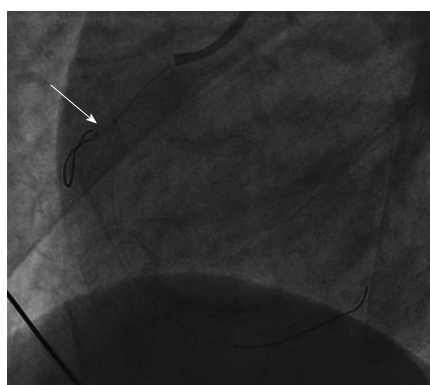


Figure 4 Same view as Figure 3 (right anterior oblique), showing balloon inflation in proximal right ventricular branch (white arrow).

Pre-discharge 12-lead ECG (Figure 1C) showed partial resolution of the ST segment elevation in lead V1 and complete resolution of ST-elevation in V4R and V5R (Figure 1D). After 4 wk, the patient was followed up with a high dose dobutamine stress echocardiography with intravenous ultrasonic contrast (SonoVue, Bracco). It showed good contractile reserve in the RV free wall and all LV segments. On this basis, we elected not to treat the residual PDA disease.

DISCUSSION

Significant RV infarction usually occurs with occlusion of the RCA, when it is accompanied by concomitant infarction of the LV segments supplied by the RCA. Smaller RV infarcts can occur with occlusion of the left circumflex and left anterior descending arteries. Involvement of the RV myocardium is common in infarction of the left ventricle. Conversely, IRVI is rare, accounting for less than 3% of all infarctions^[1].

In patients with inferior MI, the presence of RV infarction is associated with a higher risk of arrhythmias and cardiogenic shock and death. Interestingly IRVI appears to have a relatively good long term prognosis, although immediate complications resulting in sudden death have been reported^[2]. RV function tends to recover over the months following the acute event. This benign clinical course is most likely due to the thin muscular wall of this ventricle which provides a more favourable supply/demand ratio and the ample collateral supply from LV territory.

Isolated RV infarction has previously been attributed to occlusion of a non-dominant RCA or occlusion of an acute marginal artery, spontaneously or after PCI.

IRVI in the absence of the dominant current of injury from the LV posterior wall can present as ST elevation in the precordial leads V1-V3, similarly to an anterior MI. Obtaining right sided ECG may help to make this important distinction. ST elevation and Q waves in V4R is highly specific for IRVI. The ST elevation in the left precordial leads up to V5 are "dome-shaped"^[3-5].

Echocardiographic features such as RV free wall hypokinesia, dilated RV chamber, leftward septal deviation provide a valuable non-invasive diagnostic aid in acute RV infarction. Cardiac magnetic resonance provides a more accurate assessment of RV size, ejection fraction and regional wall motion abnormalities. The MR pulse sequences applied to the LV can also be used to assess the area at risk and extent of infarction by T2 weighted oedema imaging and T1 weighted gradient echo late gadolinium enhancement images, respectively.

We believe PCI is a good option for IRVI when there

is haemodynamic compromise. Management should also include strict haemodynamic monitoring, avoidance of vasodilators and diuretics, inotropic support and treatment of arrhythmias.

COMMENTS

Case characteristics

A 58-year-old male presented with sudden onset epigastric pain radiating into both arms with no significant past medical history.

Differential diagnosis

Myocardial infarction, myo-pericarditis, aortic dissection, gastritis

Laboratory diagnosis

Troponin I on admission was 1115 ng/L (normal 0-59 ng) and the peak 12-h Troponin I was 16628 ng/L.

Imaging diagnosis

Electrocardiography showed ST-elevation in lead V1 and with right ventricular leads, ST-elevation was seen in V4R and V5R leading to the diagnosis of isolated right ventricular infarction. Echocardiography showed impaired right ventricular (RV) systolic function. Coronary angiography showed occlusion of the right ventricular branch of the right coronary artery (RCA) (TIMI 0).

Treatment

The patient was given aspirin 300 mg, ticagrelor 180 mg, atorvastatin 80 mg before transfer for urgent primary percutaneous intervention. Balloon angioplasty was performed at the proximal occlusion in the right ventricular branch of the RCA. Bivalirudin give peri-procedure.

Related reports

Reported cases comment on RV branch involvement with the main RCA and isolated occlusion usually as a complication of percutaneous intervention to the RCA. Anecdotal evidence and received wisdom favour a conservative approach, with the belief this small vessel can be sacrificed without little harm. However, each case demands a nuanced approach with an individualized risk-

benefit analysis. An isolated branch occlusion can occur and can have dire consequences without prompt intervention.

Experiences and lessons

This is a rare case report that teaches the authors' to be "exsisto semper vigilans-to be ever vigilant". It is an infrequent case in their daily clinical practice that may be easily missed leading to a delay in diagnosis and management, which may lead to fatal consequences.

Peer review

This is a well written case report that offers useful clinical information.

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Volume End



**EDITORIAL**

- 1227 Etiology of bicuspid aortic valve disease: Focus on hemodynamics
Atkins SK, Sucosky P

TOPIC HIGHLIGHT

- 1234 Arrhythmogenic right ventricular cardiomyopathy: From genetics to diagnostic and therapeutic challenges
Pinamonti B, Brun F, Mestroni L, Sinagra G
- 1245 Experimental models of inherited cardiomyopathy and its therapeutics
Nonaka M, Morimoto S

REVIEW

- 1252 Arginine vasopressin as a target in the treatment of acute heart failure
Gilotra NA, Russell SD

MINIREVIEWS

- 1262 Implications of Klotho in vascular health and disease
Martín-Núñez E, Donate-Correa J, Muros-de-Fuentes M, Mora-Fernández C, Navarro-González JF
- 1270 Cardiac resynchronization therapy: Dire need for targeted left ventricular lead placement and optimal device programming
Pastromas S, Manolis AS

CASE CONTROL STUDY

- 1278 Complicated Whipple's disease and endocarditis following tumor necrosis factor inhibitors
Marth T

CASE REPORT

- 1285 Dangerous triplet: Polycystic ovary syndrome, oral contraceptives and Kounis syndrome
Erol N, Karaagac AT, Kounis NG
- 1290 Spontaneous coronary artery dissection as a cause of myocardial infarction
Aksakal A, Arslan U, Yaman M, Urumdas M, Ates AH

Contents

World Journal of Cardiology
Volume 6 Number 12 December 26, 2014

APPENDIX I-V Instructions to authors

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Etiology of bicuspid aortic valve disease: Focus on hemodynamics

Samantha K Atkins, Philippe Sucosky

Samantha K Atkins, Philippe Sucosky, Department of Aerospace and Mechanical Engineering, University of Notre Dame, Notre Dame, IN 46556, United States

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Correspondence to: Philippe Sucosky, PhD, FAHA, Department of Aerospace and Mechanical Engineering, University of Notre Dame, 143 Multidisciplinary Research Building, Notre Dame, IN 46556-5637, United States. philippe.sucosky@nd.edu
Telephone: +1-574-6311898 Fax: +1-574-6312144

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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

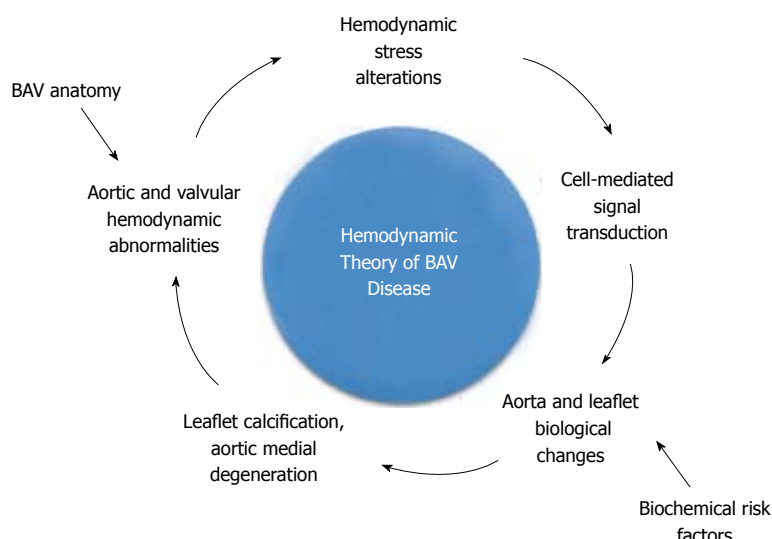


Figure 1 Hemodynamic theory of bicuspid aortic valve disease. The hemodynamic theory of bicuspid aortic valve (BAV) disease can be illustrated as an irreversible feedback loop in which the BAV anatomy would subject the valve leaflets and the ascending aortic wall to local stress overloads. Those stress 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

Bruno Pinamonti, Francesca Brun, Luisa Mestroni, Gianfranco Sinagra

Bruno Pinamonti, Francesca Brun, Gianfranco Sinagra, Cardiovascular Department, Ospedali Riuniti of Trieste, 34100 Trieste, Italy

Luisa Mestroni, Cardiovascular Institute and Adult Medical Genetics Program, University of Colorado Denver AMC, Aurora, CO 80045-2507, United States

Author contributions: Pinamonti B and Brun F contributed equally to the writing of this review article; all the authors contributed to this work.

Correspondence to: Bruno Pinamonti, MD, Cardiovascular Department, Ospedali Riuniti of Trieste, via P. Valdoni n° 7, 34100 Trieste, Italy. bpinamonti@hotmail.com

Telephone: +39-040-3994878

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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

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

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

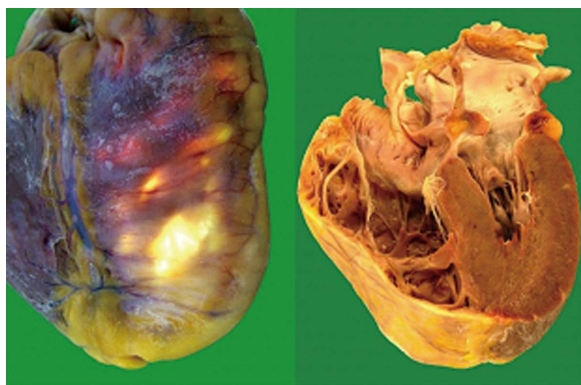


Figure 1 Gross anatomic specimens in a patient affected by arrhythmogenic right ventricular cardiomyopathy who died suddenly. Severe right ventricular enlargement and wall atrophy and fatty replacement are evident.

(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,

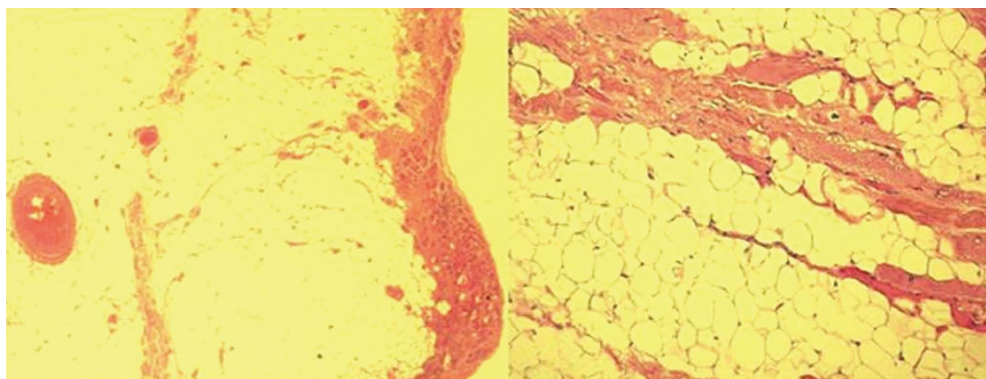


Figure 2 Histologic specimens of a case with arrhythmogenic right ventricular cardiomyopathy that show severe right ventricular fibro-fatty replacement and loss and degeneration of myocytes (Hematoxylin Eosin $\times 2.5, \times 10$).

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%^[24,25]. The most frequently affected gene is *PKP2* with a reported detection rate of 10%-45%^[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

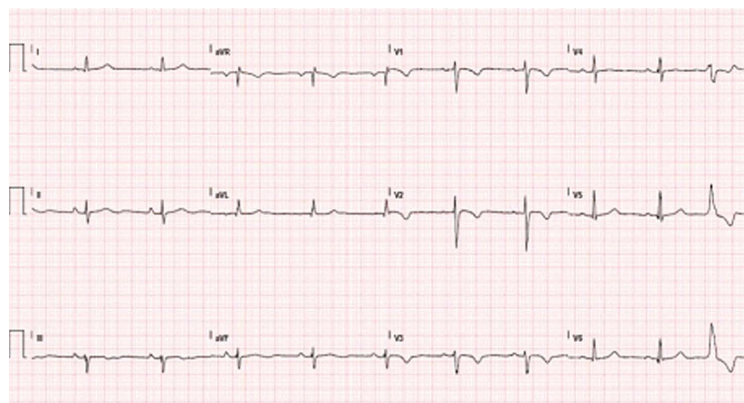


Figure 3 Typical electrocardiogram in a patient with classical arrhythmogenic right ventricular cardiomyopathy. Negative T waves in anterior precordial leads are present. A ventricular premature beat with left bundle branch block morphology is also observed.

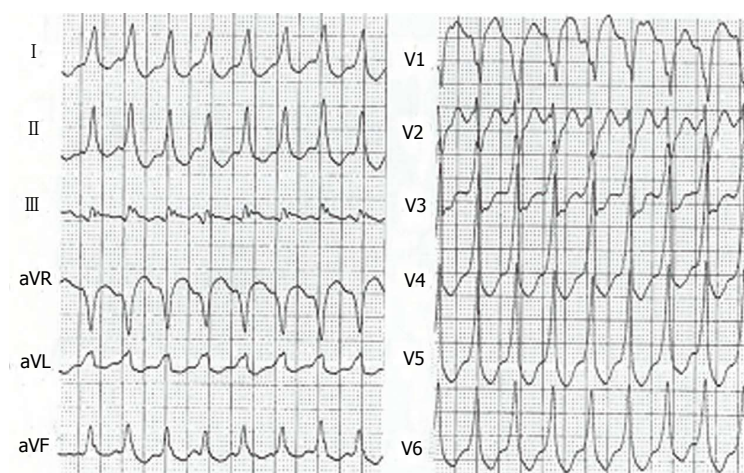


Figure 4 Ventricular tachycardia with left bundle branch block pattern in a patient with arrhythmogenic right ventricular cardiomyopathy.

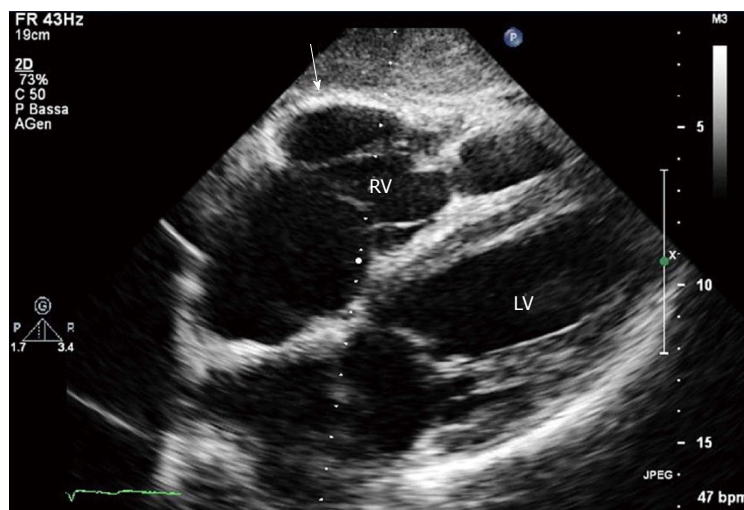


Figure 5 Two-dimensional echocardiogram, 4 chamber subcostal view, end diastolic frame, in an arrhythmogenic right ventricular cardiomyopathy patient. RV wall aneurysm at subtricuspid basal level is evident (arrow). LV: Left ventricle; RV: Right ventricle.

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

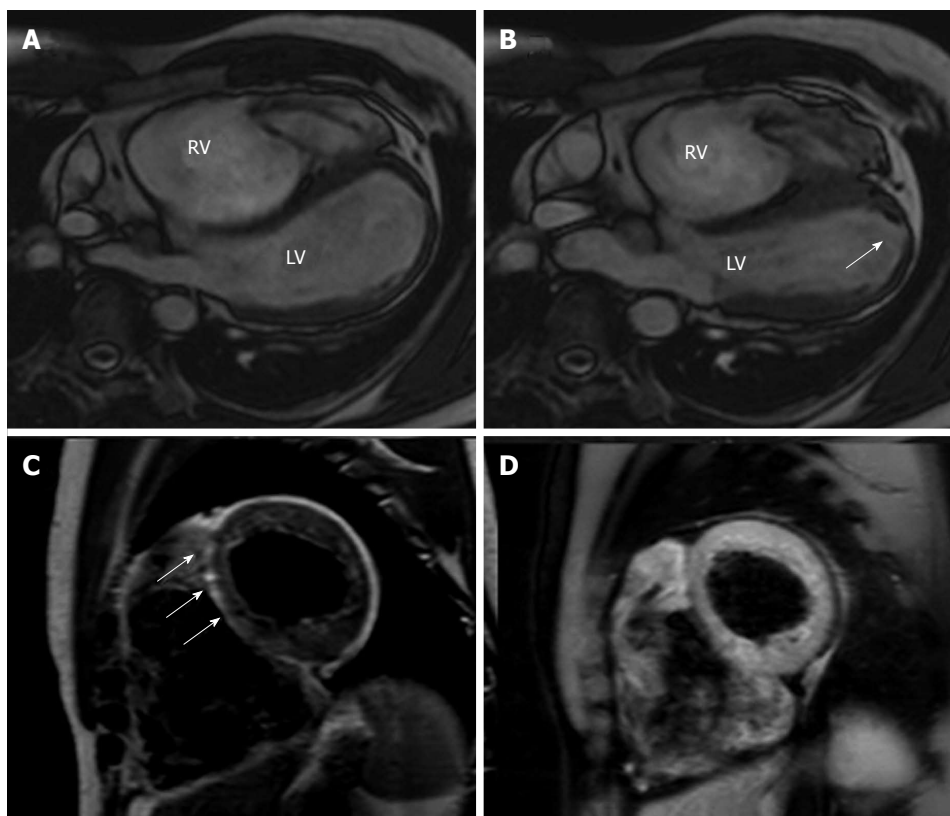


Figure 6 Cardiac magnetic resonance images of an arrhythmogenic right ventricular cardiomyopathy patient with mild left ventricular involvement. A, B: End-diastolic and end-systolic frames, off-axis 4 chamber view. Note the multiple bulging segments at right ventricular (RV) free wall. An apical hypokinesia 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

Miki Nonaka, Sachio Morimoto

Miki Nonaka, Sachio Morimoto, Department of Clinical Pharmacology, Kyushu University Graduate School of Medicine, Fukuoka 812-8582, Japan

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Correspondence to: Sachio Morimoto, PhD, Department of Clinical Pharmacology, Kyushu University Graduate School of Medicine, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. morimoto@med.kyushu-u.ac.jp

Telephone: +81-92-6416081 Fax: +81-92-6426084

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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 gene 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

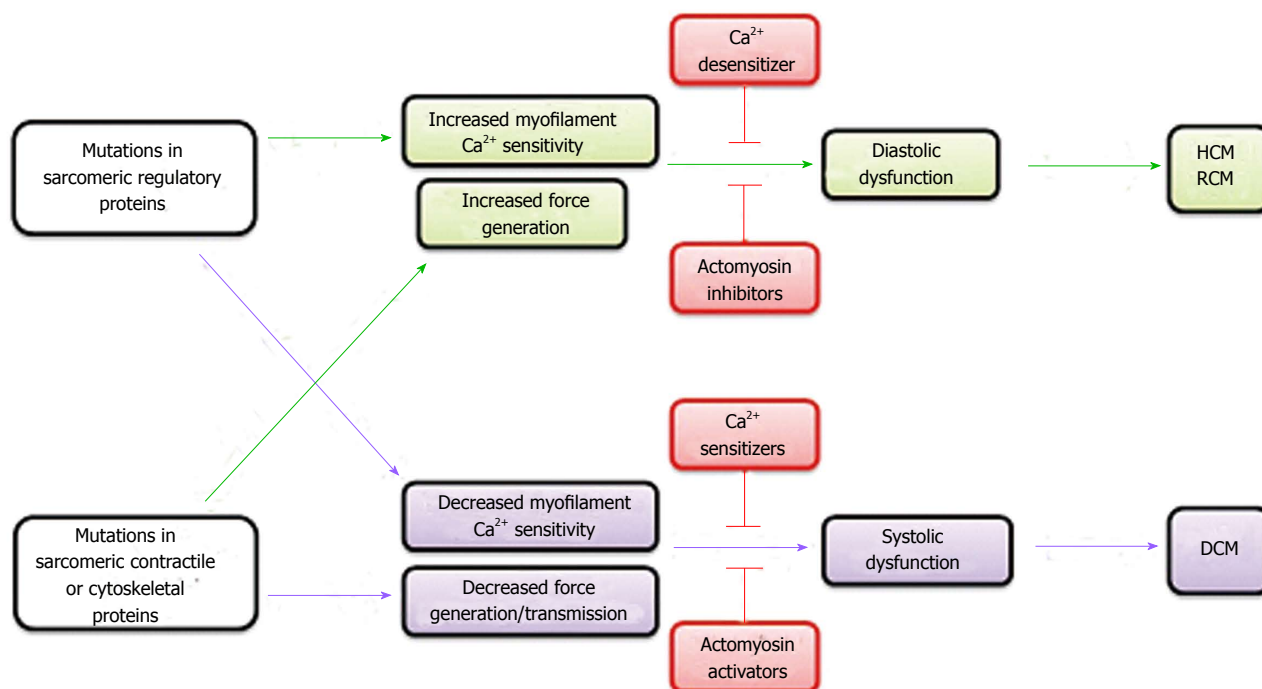


Figure 1 Essentials of pathogenic mechanisms in inherited cardiomyopathies and potential definitive drug therapies. HCM: Hypertrophic cardiomyopathy; DCM: Dilated cardiomyopathy; RCM: Restrictive cardiomyopathy.

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

Nisha A Gilotra, Stuart D Russell

Nisha A Gilotra, Stuart D Russell, Division of Cardiology, Department of Medicine, Johns Hopkins Hospital, Baltimore, MD 21287, United States

Author contributions: Gilotra NA and Russell SD contributed equally to this work.

Correspondence to: Stuart D Russell, MD, Associate Professor of Medicine, Chief, Advanced Heart Failure and Transplant, Division of Cardiology, Johns Hopkins Hospital, 1800 Orleans Street, Sheikh Zayed Tower, Suite 7125, Baltimore, MD 21287, United States. srusse14@jhmi.edu

Telephone: +1-410-9555708 Fax: +1-410-9553478

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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

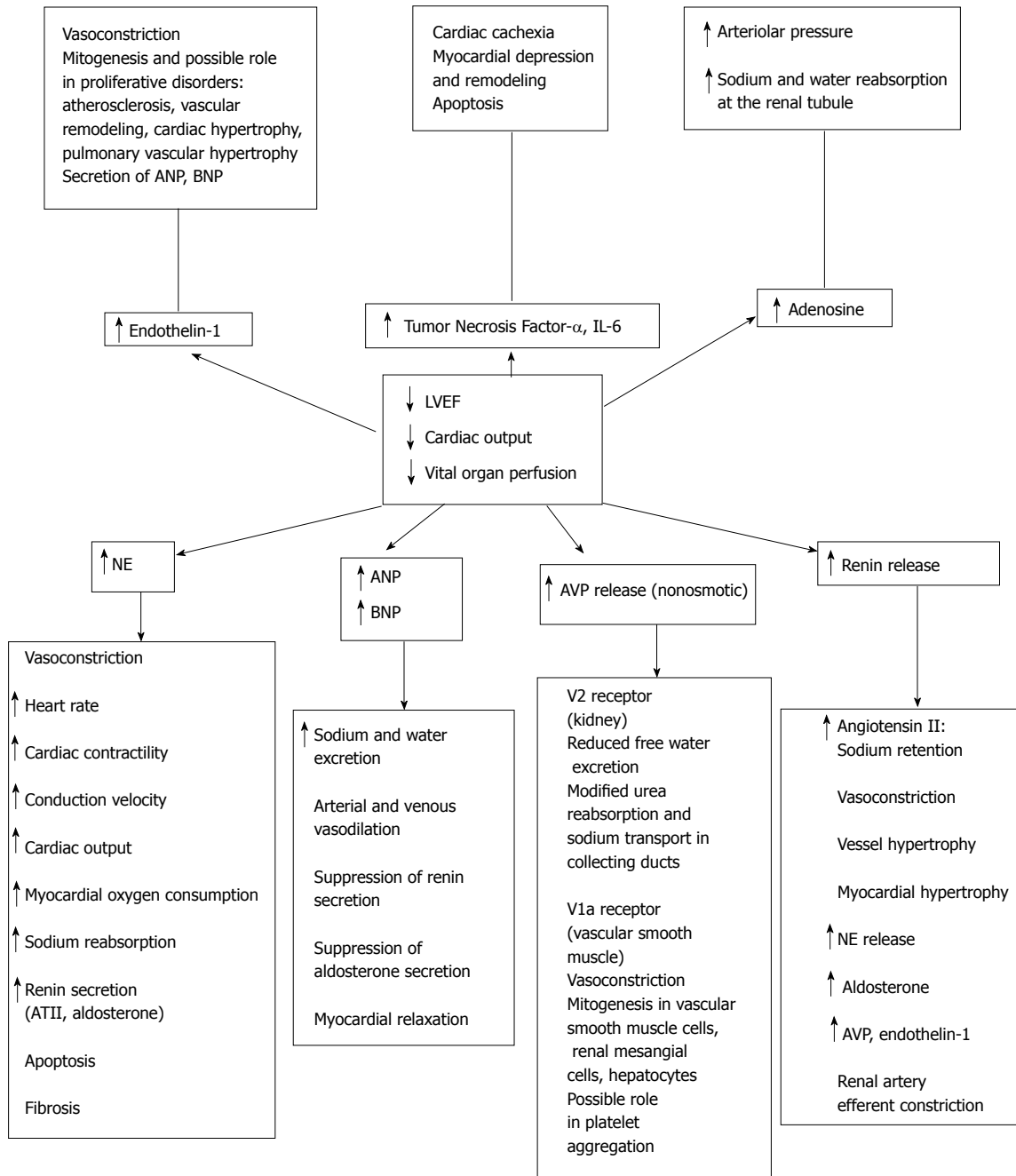


Figure 1 Summary of neurohormonal activation in heart failure. With injury to the left ventricle and subsequent decrease in left ventricular function, there is a decrease in cardiac output, subsequent decrease in perfusion of vital organs, and activation of the various neurohormonal systems. ANP: Atrial 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 euvolemic 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 euvolemic hyponatremia. It is currently approved for treatment of euvolemic 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

Ernesto Martín-Núñez, Javier Donate-Correa, Mercedes Muros-de-Fuentes, Carmen Mora-Fernández,
 Juan F Navarro-González

Ernesto Martín-Núñez, Javier Donate-Correa, Carmen Mora-Fernández, Research Unit, University Hospital Nuestra Señora de Candelaria, 38010 Santa Cruz de Tenerife, Spain
 Mercedes Muros-de-Fuentes, Clinical Analysis Service and Research Unit, University Hospital Nuestra Señora de Candelaria, 38010 Santa Cruz de Tenerife, Spain
 Juan F Navarro-González, Nephrology Service and Research Unit, University Hospital Nuestra Señora de Candelaria, 38010 Santa Cruz de Tenerife, Spain

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Correspondence to: Juan F Navarro-González, MD, PhD, Research Unit, University Hospital Nuestra Señora de Candelaria, Carretera del Rosario, 38010 Santa Cruz de Tenerife, Spain. jnavgon@gobiernodecanarias.org

Telephone: +34-922-600566 Fax: +34-922-600562

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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),

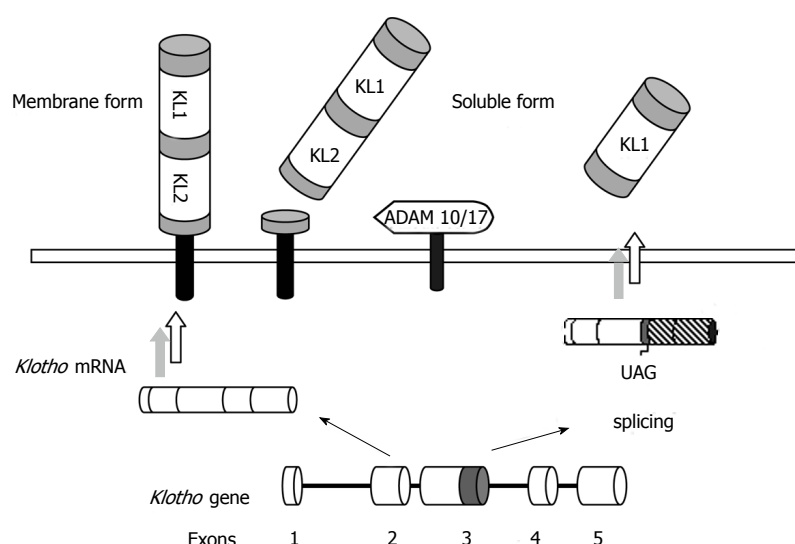


Figure 1 Mechanisms of generation of the different forms of Klotho. ADAM: Membrane-anchored A Disintegrin and metalloproteinase.

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 Disintegrin 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

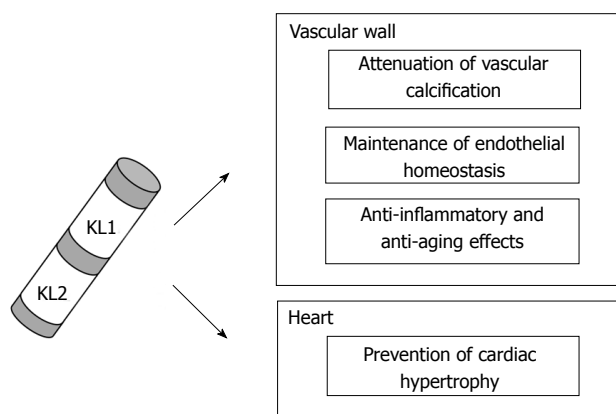


Figure 2 Mechanisms of vascular protection mediated by Klotho.

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

Sokratis Pastromas, Antonis S Manolis

Sokratis Pastromas, Antonis S Manolis, Department of Cardiology, Evagelismos Hospital, and Athens University School of Medicine, 10676 Athens, Greece

Author contributions: Pastromas S reviewed the literature, composed and wrote the article; Manolis AS conceived the topic and the title, made suggestions, provided literature, critically reviewed and edited the article, made corrections and guided the initial submission and revision process.

Correspondence to: Antonis S Manolis, MD, Department of Cardiology, Evagelismos Hospital, and Athens University School of Medicine, Ypsilantou 45-47, 10676 Athens, Greece. asm@otenet.gr

Telephone: +30-213-2041493 Fax: +30-213-2041495

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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,

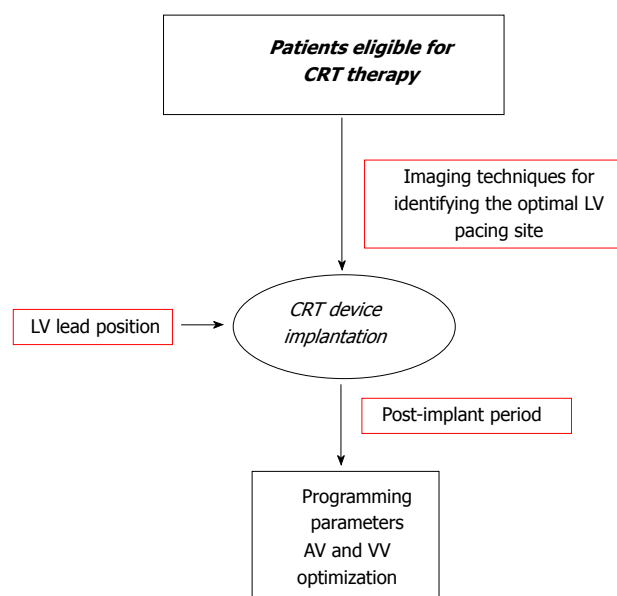


Figure 1 The factors that interact for the cardiac resynchronization response and improvement of heart failure symptoms. AV: Atrioventricular; CRT: Cardiac resynchronization; LV: Left ventric-le(-ular); VV: Interventricular.

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

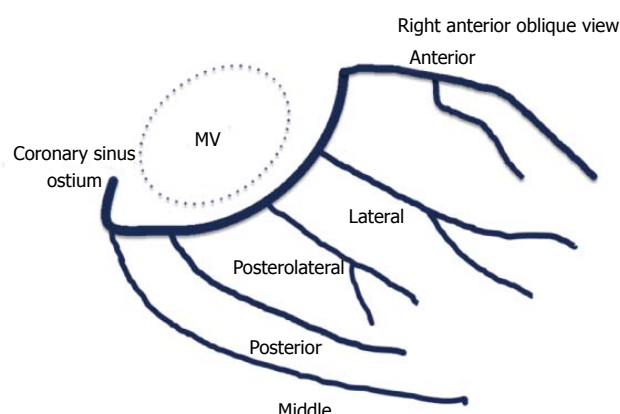


Figure 2 Coronary sinus anatomy as illustrated in the right anterior oblique view used to identify the optimal posterior, lateral and posterolateral branches for the left ventricular lead placement. MV: Mitral valve.

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

Thomas Marth

Thomas Marth, Division of Internal Medicine, Krankenhaus Maria Hilf, 54550 Daun, Germany

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Correspondence to: Thomas Marth, MD, Professor of Internal Medicine, Division of Internal Medicine, Krankenhaus Maria Hilf, Maria-Hilf-Straße 2, 54550 Daun, Germany. t.marth@krankenhaus-daun.de

Telephone: +49-6592-7152221 Fax: +49-6592-7152501

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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, adalimu-mab/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, adalimu-mab/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-gamma 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. whippelii* 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. whippelii* 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 whippelii* (*T. whippelii*), 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. whippelii* infection.

Innovations and breakthroughs

This study shows that treatment with TNFI may trigger severe complications of *T. whippelii* infection, particularly endocarditis. Medical immuno-suppression plays a role in the progression of asymptomatic *T. whippelii* 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. whippelii* 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

Nurdan Erol, Aysu Turkmen Karaagac, Nicholas G Kounis

Nurdan Erol, Siyami Ersek Thorax and Cardiovascular Surgery Training and Research Hospital, Pediatric Cardiology Clinic, 34668 Uskudar, Istanbul, Turkey

Aysu Turkmen Karaagac, Kartal Kosuyolu Research and Training Hospital Pediatri, 34846 Kartal, Istanbul, Turkey

Nicholas G Kounis, Department of Medical Sciences, Northwestern Greece Highest Institute of Education and Technology, 26221 Achaia, Greece

Author contributions: Erol N carried out acquisition of data, interpreted the results and wrote the article; Kounis NG contributed to analysis and interpretation of data and revising the paper; Karaagac AT contributed to write the article.

Correspondence to: Nurdan Erol, MD, Pediatric Cardiologist, Siyami Ersek Thorax and Cardiovascular Surgery Training and Research Hospital, Pediatric Cardiology Clinic, Tibbiye Caddesi No:13, 34668 Uskudar, Istanbul, Turkey. dmurdanerol@superonline.com

Telephone: +90-505-7588787 Fax: +90-216-5424444

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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

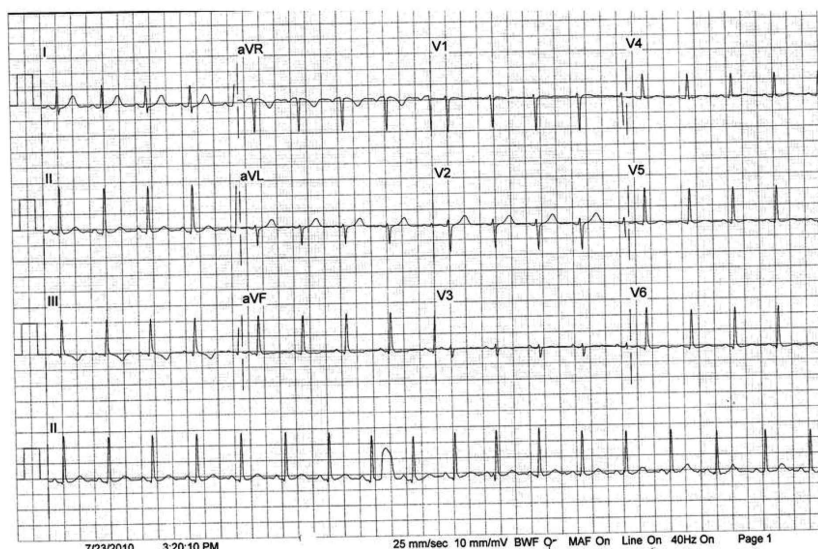


Figure 1 Electrocardiogram during chest pain with increased troponin showing T wave inversion in lead III and flattening of T wave in lead left foot derivation in electrocardiography.

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,

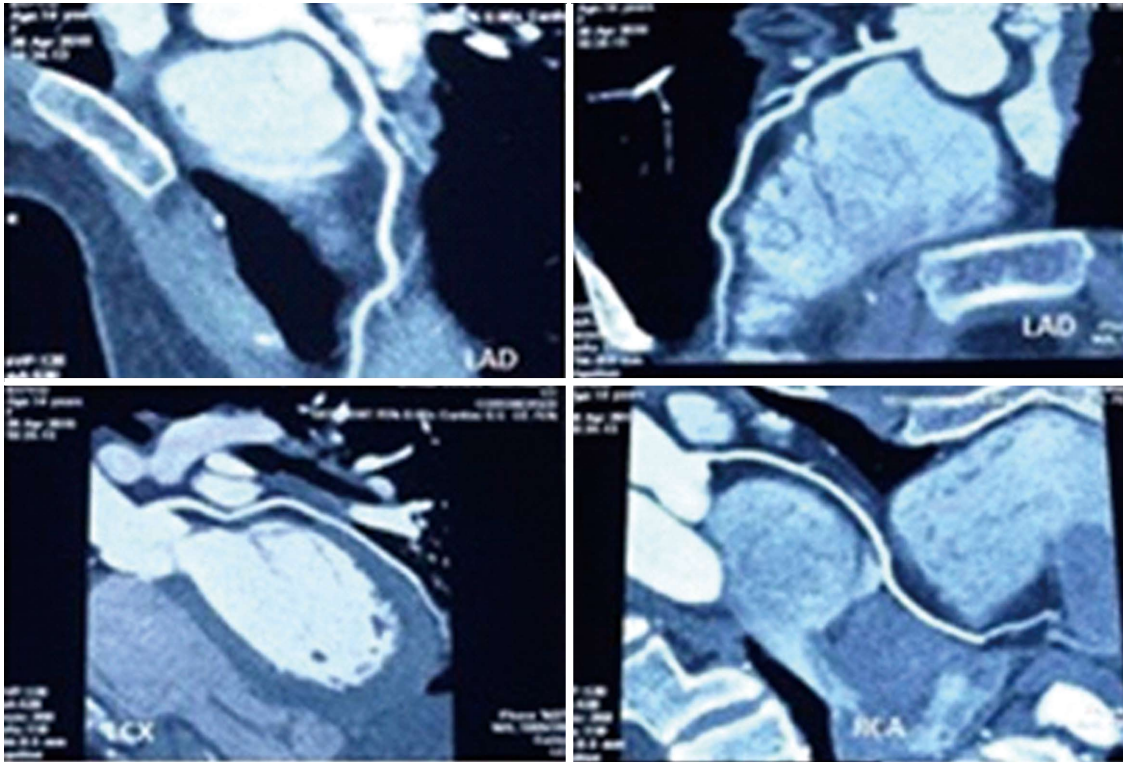


Figure 2 Normal computed tomography angiogram excluding thrombosis.

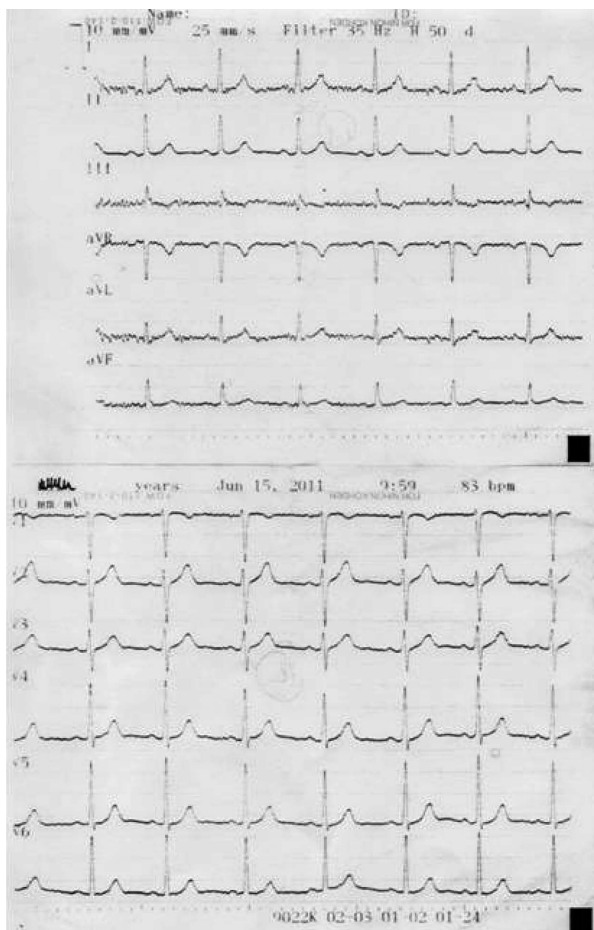


Figure 3 Normal electrocardiogram during cessation of contraceptive while the eosinophils were increased.

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

Aytekin Aksakal, Uğur Arslan, Mehmet Yaman, Mehmet Urumdaş, Ahmet Hakan Ateş

Aytekin Aksakal, Uğur Arslan, Mehmet Yaman, Mehmet Urumdaş, Ahmet Hakan Ateş, Department of Cardiology, Samsun Education and Research Hospital, Samsun 55400, Turkey

Author contributions: Aksakal A and Arslan U designed the case report; Yaman M and Ateş AH performed the coronary angiography and stenting procedures of the patient and searched for the references; Aksakal A, Arslan U and Urumdaş M wrote and revised the paper.

Correspondence to: Uğur Arslan, MD, Associate Professor, Department of Cardiology, Samsun Education and Research Hospital, Samsun 55400, Turkey. ugurarslan5@yahoo.com
Telephone: +90-532-6039983 Fax: +90-362-2778569

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Key words: Coronary artery dissection; Myocardial bridge; Myocardial infarction; Coronary artery disease; Acute coronary syndrome

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>
DOI: <http://dx.doi.org/10.4330/wjc.v6.i12.1290>

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

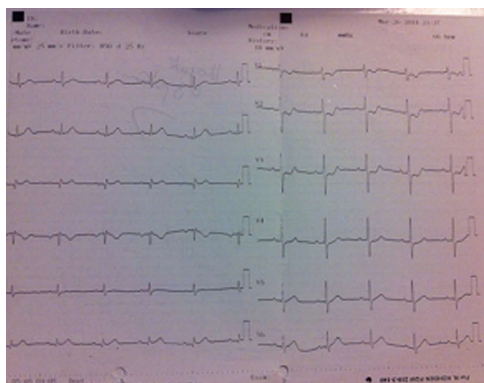


Figure 1 Electrocardiogram showing ST depression in leads V1-V3.

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

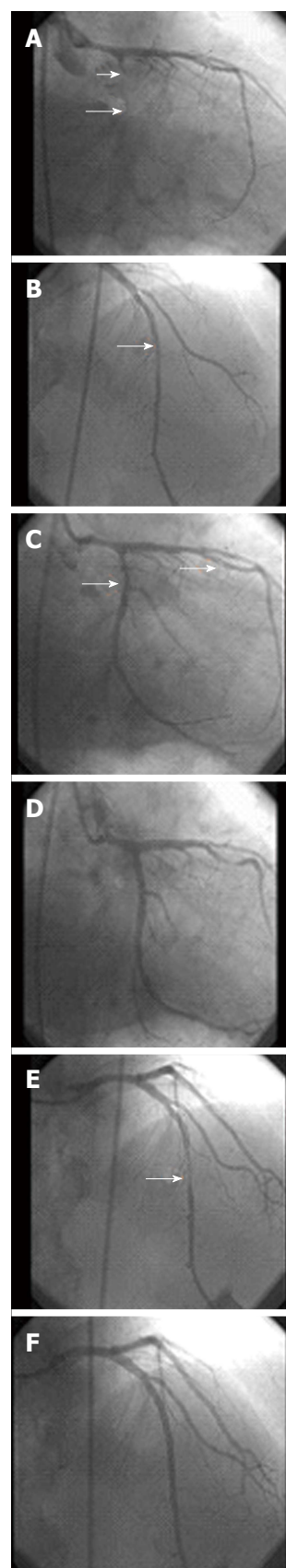


Figure 2 Coronary angiographic views of the patient. The arrows indicate left circumflex (LCx) total occlusion and proximal dissection in (A), coronary dissection in left anterior descending (LAD) (B), simultaneous coronary dissection in LAD and LCx arteries in (C) and subtotal occlusion and dissection 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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